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A STUDY OF THE ORGANISMS OCCURRING IN TWO CASES OF EROSIO INTERDIGITALIS*

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EROSIO INTERDIGITALIS is considered to be a rare disease, but probably occurs more frequently in America than the literature indicates. The clinical characteristics and the ease with which the organism may be found in the scale make the diagnosis comparatively easy.

The two cases from which the material for our studies was secured, occurred in a series of 450 cases at the Skin Clinic of the St. Louis City Dispensary.

Both patients were elderly Jewish women of Russian nativity. Each did her own housework, including the washing of dishes and clothes. Soap was used by both patients in the clothes washing and general cleaning but not in the washing of dishes.

REPORT OF CASES

CASE 1.—*History.*—On July 22, 1924, Mrs. F. E., aged sixty-four years, presented herself at the clinic complaining of itching lesions between the fingers. The lesions had been present for ten months, and were located on the web of the third interspace of the right hand, and the third and fourth interspaces of the left hand. All lesions had developed at the same time, progressed to a certain point, and had then remained stationary.

Subjective symptoms.—Intense itching at times.

Objective symptoms.—The lesions were sharply defined, superficial and confined to the web of fingers. They had never extended to the palm or dorsum of the hand. They were covered with a loose layer of white, sodden epidermis, which could be removed easily, without discomfort to the patient. When this was done, there was disclosed a smooth, shiny, red

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epidermis, without fissures, vesicles or evidence of exudation. The patient's general health was good. No other dermatologic lesions were present. (Figs. 1 and 2.)

CASE 2.—*History.*—Mrs. I. S., aged sixty years. Born in Russia, but a resident of America for eighteen years. This patient was first seen at the Skin Clinic of the St. Louis City Dispensary on August 7, 1924. She presented a lesion on the web of the third interdigital space of both hands. The one on the right hand had been present for four years; the one on the left for a year and a half. As in the first case, the lesions had neither extended nor retrogressed since the onset, and had remained confined to the webs of the fingers.

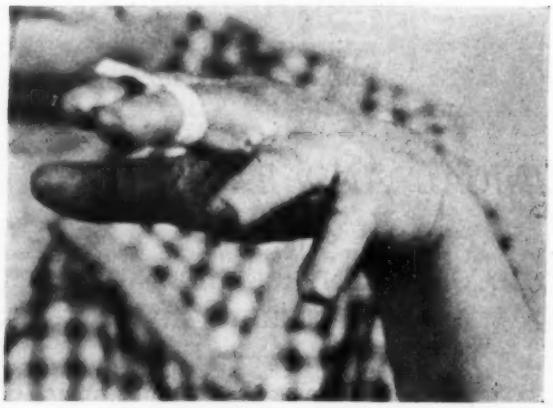

Fig. 1.—Shows the lesion on the left hand of patient "F.E."

Fig. 2.—Shows the lesions on the right hand of patient "F.E."

Subjective symptoms.—Occasional slight itching.

Objective symptoms.—At the time of the first examination the lesions compared very closely with those described by Mitchell.[1] They were dry, shiny, red, and surrounded by a "collarette of scales," which were removed with difficulty and the removing caused considerable pain to the patient. When, however, the patient returned one week later, on the day following her wash day, the red surface was covered with a white, sodden epidermis which could be removed without pain. There were no other dermatologic lesions present. (Fig. 3.)

Microscopic examination.—The scales of both cases when treated with potassium hydroxide solution revealed many round and oval double contoured organisms.

The literature pertaining to studies of the organisms isolated from so-called cases of Blastomycosis interdigitale or Intertrigo saccharomycetica, or Erosio interdigitalis blastomycetica seu saccharomycetica, does not furnish very satisfactory evidence concerning the organisms actually dealt with by the various authors. In general, most of the authors refer to the organisms found in these types of skin lesions (whether they occur as interdigital lesions or elsewhere on the skin of other parts of the body) as yeasts, yeast-like organisms or blastomyces. Possibly a review of the earlier studies of the yeast-like organisms causing lesions of the skin or occurring in the skin will be of interest.

It has been noted by a number of observers that in cases of thrush, organisms similar to or identical with those causing the lesions in the mouths of the infants can at times be found in skin lesions and in the feces of such cases. Thus in 1907, Moro[2] noted the occurrence of so-called "soorpilze" in

Fig. 3.—Shows the lesions on both hands of patient "I.S."

skin lesions about the anus of children suffering with thrush and he further found the "endomyces" in the feces of such cases. Chiray and Sartory[3] found similar organisms in the stools of poorly nourished infants. Ibrahim[4] found similar organisms about the anus, and in lesions of the skin, both intertriginous (between the fingers or toes) and nonintertriginous—which organisms he did not, however, definitely consider to be the causative agents of the disease.

Whitfield,[5] who reported a case of intertrigo in 1908, simply called the organism demonstrated a yeast, and apparently made no further effort to identify the organisms.

Gougerot and Goncia,[6] who reported a case of intertrigo in 1914, describe the organism as being like that found in thrush but not the Endomyces albicans. They describe the culture of the organism on solid media as creamy and raised; they studied the cultural qualities on various media, and also made a study of the agglutinative and complement-fixation qualities of the organism as compared to Endomyces albicans and Sporotrichum beurmanni

and Sporotrichum gougeroti. They describe their organism as spherical or ovoid and showing budding. They call it a yeast-like organism but go no further in their identification. Since they saw no asci or ascospores, their organism cannot be classed as a saccharomyces.

Hudelo and Montlaur,[7] and later Hudelo, Sartory and Montlaur[8] reported a number of cases in which they isolated from the skin lesions (usually intertriginous) yeast-like organisms, which, in the later article, are classed as saccharomyces. In the scales they demonstrated oval cells arranged in filaments. The spherical to ovoid organisms showed budding at one and occasionally at both poles, and they were able to demonstrate asci and four ascospores in the sac. The cultures showed pseudomycelia at times but no chlamydospores. The growth was creamy, and in color white to "café au lait." The organism fermented maltose but had no action on lactose, galactose or levulose, and did not liquefy gelatin.

They also found a similar organism, identical in cultural and biochemic qualities, but showing no asci or ascospores, with more abundant pseudomycelia and much like Endomyces albicans. Whether these latter were found associated with the saccharomyces in the lesions, or whether the two types were found in lesions from different cases is not clear from their report.

Fabry[9] and his coworkers state that yeasts were found in cases of Erosio interdigitalis blastomycetica. The description of the morphology and cultural qualities of the organisms by Berendsen[10] is quite inadequate to permit of assigning these organisms to any specific genus or even suggesting their biologic classification.

Tanner and Feuer[11] described and studied quite thoroughly an organism isolated from an infection of the skin. The organism was round to ovoid, 3.5 to 6.5 μ and showed no spore forms. It grew on glucose agar as a white, slimy, luxuriant growth; coagulated and digested casein but did not render it acid, and fermented glucose completely, but did not act upon sucrose. They classified the organism as an Endomyces albicans.

Engman[12] in 1920 described an infection of the skin due to an organism which he classed as a hyphomycetes or monilias—as either a monilia or oidium and belonging to the genus Botrytis. Unfortunately, he does not give a description of the cultural characteristics of the organism, but evidently depends upon authoritative advice for the identification.

Castellani and Chalmers,[13] in their *Manual of Tropical Medicine,* mention as potential causes (agents) in Intertrigo saccharomycetia both saccharomyces and also fungi of the genus monilia.

Stechel[14] in 1921 speaks of the organisms found in interdigital erosions as yeasts, but gives no cultural description nor definite basis for this nomenclature.

Weidman[15] calls attention to the morphologic characters noted in the organisms in skin scrapings from lesions between the fingers, toes and about the anus, and states that these grew as yeasts but showed mycelia in old glucose agar plates. One of the photographs of the organisms shows an appearance practically identical with the typical monilia (or soorpilze) growth

form. He writes of the resemblance of yeasts to hyphomycetes, so presumably he classes these organisms as yeasts.

Mitchell,[16] writing on Erosio interdigitalis blastomycetica, does not consider specifically the organisms causing this type of lesion.

Greenbaum and Klauder[17] separate the yeasts causing intertriginous infections of the skin into four types, and we give below their tabulation of the points of differentiation as they have stated them:

	TYPE I	TYPE II	TYPE III	TYPE IV
Glucose Agar				
Surface	Wrinkled	Smooth	Smooth	Smooth
Color	Cream	White	Yellow	Salmon
Raulin Media	Sediment	Sediment	Sediment	Sediment
Glucose	0	0	0	+
Levulose	0	0	0	0
Maltose	0	0	0	0
Mannite	0	0	0	0
Litmus	Acid	Acid	Acid	Amphoteric

They classify them all excepting the second type as saccharomyces and this exception they classify as a cryptococcus. Ascospores or asc sacs were demonstrated in Types I, III and IV. In studying yeasts from normal skin they found these same types present in a number of individuals; thus in 35 of 150 individuals examined, one or another of these four types of yeasts were demonstrated in culture. In a series of seven interdigital lesions these same types of organisms were present; thus Type I and Type II each in two cases, and Type III in three cases.

It would appear then from this review of the literature that: First, a large part of the work concerning so-called yeasts as agents in causing diseases of the skin is inadequate to permit of drawing any conclusion as to the actual type of organisms causing the disease. Second, that apparently true saccharomyces (producing asci and ascospores) may be the causative agents in intertriginous conditions, as shown by Hudelo, Sartory and Montlaur, and by Greenbaum and Klauder. Third, that in infants affected with thrush apparently the same organisms (endomyces or monilia or oidia) which cause the lesions of the mouth may also cause intertriginous lesions. Fourth, that apparently monilia or similar organisms may cause lesions of the skin (intertriginous or otherwise), even when there is no thrush, as shown in the reports of Castellani and Chalmers, Tanner and Feuer, and Engman.

MYCOLOGIC STUDIES

We have isolated and studied organisms (fungi) from the skin scrapings (scales) of both of the above cases, and have also isolated either similar or identical organisms from the stools in both cases. Thus in the case I. S., the two organisms (No. 203 skin and No. 203 stool) are apparently identical, while in the case F. E. there seem to be slight differences between the organism isolated from the skin (No. 201 skin) and that from the stool (No. 201 stool). The two organisms isolated from the skin are very similar, although probably not identical. Thus the skin organisms from I. S. and F. E. and the stool

organism from I. S. are all very similar, while the stool organism from F. E. differs somewhat, and therefore must be described separately.

The three organisms, No. 201 skin, No. 203 skin, No. 203 stool, are spherical to ovoid, measuring about 4 to 5 μ by 2.5 to 4 μ. When stained with methylene-blue or thionin, the larger forms are unstained or show only a faint staining which is most marked at the periphery and which suggests a granular structure of the cell; otherwise the cells, stained in this manner, show little or no evidence of structures such as a nucleus or vacuoles. The smaller forms show a fairly deep staining at the periphery which tends to be more marked at the poles, or which may be confined to limited parts of the periphery, or

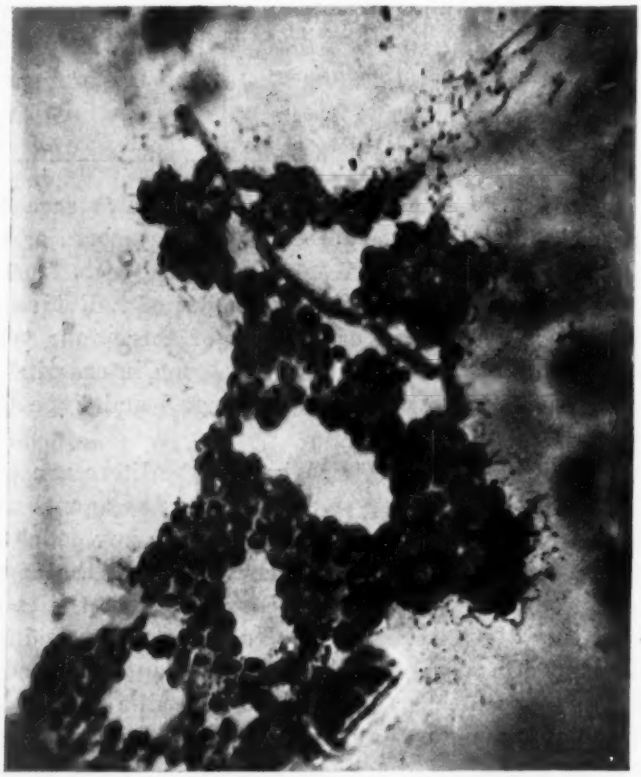

Fig. 4.—Culture No. 203 st. Stained smear from Sabouraud's media, four days' growth, showing spherical, irregularly stained cells and also a few unstained cells. Mycelium also seen. Note the similarity between the forms seen in No. 203 st. and No. 201 st. after four weeks' growth.

the staining may appear darker in the peripheral part and faint in the remainder of the cell. The variation in staining is very marked so that we may run the gamut from cells which are practically unstained to others which are quite diffusely stained in a rather uneven, granular fashion. On the whole the larger forms stain less deeply than the smaller forms. In old cultures there are apparently more of the larger, paler forms than in the young (twenty-four-hour) cultures. (Figs. 4 and 5.)

We find also a few longer, thinner cells, varying from no longer than the larger yeast-like cells to two or three times their length, and in width

usually about half the size of the yeast-like cells. These longer cells which are apparently mycelial cells stain irregularly and usually rather faintly, and show one or more refractile bodies within the cells. Usually these mycelial forms are seen in cultures from four to eight days old (Fig. 6).

In the unstained specimens we note forms essentially similar to those seen in the stained specimens. We find large cells with a small amount of granular matter at the periphery; these cells are spherical rather than round. The smaller forms are ovoid, very variable in size, show a double contoured periphery and contain one, or occasionally two bright spots (large when only one, and small when two are present). These bright spots—probably

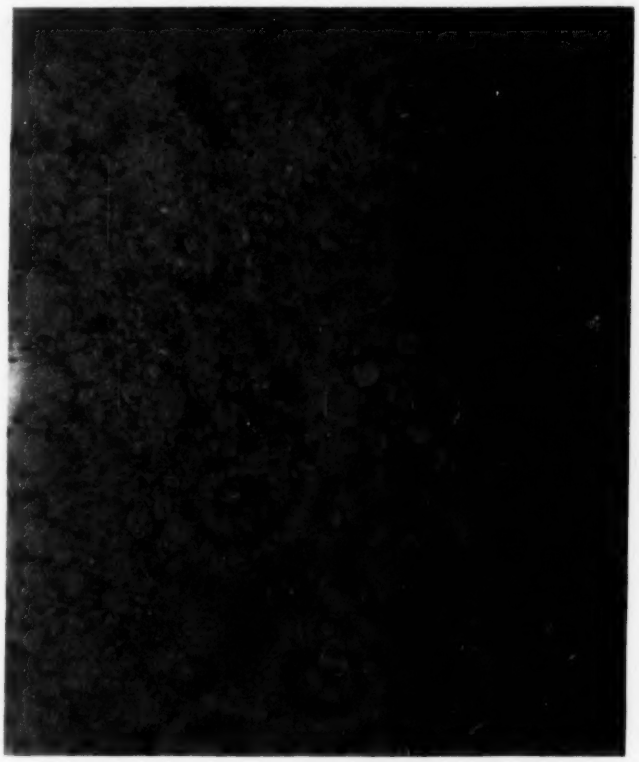

Fig. 5.—Culture No. 203 sk. Smear from Sabouraud's media. Four weeks' growth, showing densely packed masses of spherical cells which usually do not stain. Note the extreme variation in the sizes of the cells, some suggesting the so-called durable forms.

nuclei—are at times centrally located, at times lie toward one pole of the cell; they are usually round and are probably from 0.5 to 1.5 μ in diameter. Spores have not been demonstrated in any of the cultures. In each cell there may be noted a dark, actively motile granule which is present in every cell, with the possible exception of some of the very largest forms.

When the smears are fixed with Bouin's solution and stained with hematoxylin (Harris') we note a very faintly stained large, round or spherical area toward the center of the cell; occupying, in some cells, the greater part of the cell, in others, only a limited portion. These masses are usually clear in the center, or may show a few granules stained with the hematoxylin, but

show a definite peripheral staining; there is little question but that they are nuclei. In many cells several nuclei may be seen and practically always in the elongated mycelial forms of cells we find two or more nuclei. We see also, usually toward one pole of the cell, a mass or several masses, usually granular and more or less deeply stained. These lie in close contact with the nucleus and they vary very markedly in size and outline. They seem to correspond possibly with the vacuoles.

When the yeast cells are examined in hanging drop with a weak solution of neutral red, the structure which we have called the nucleus is un-

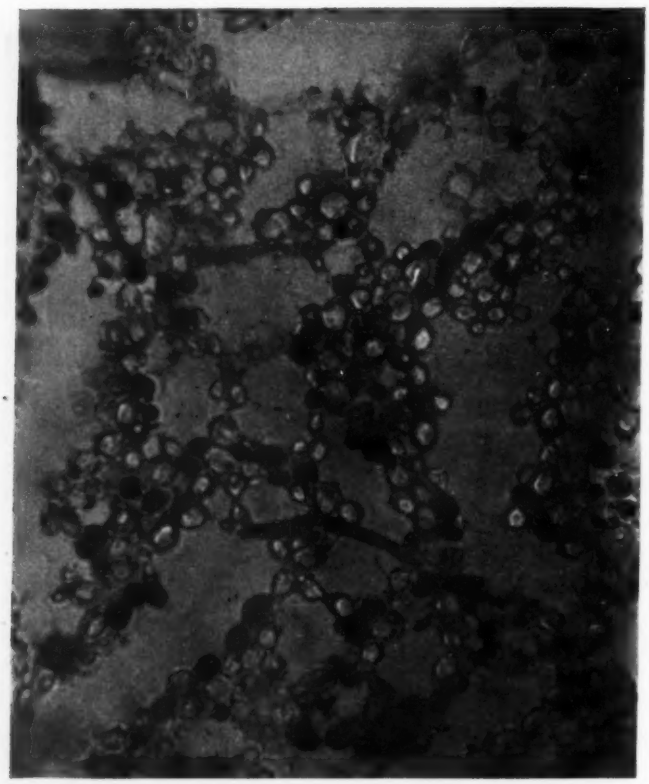

Fig. 6.—Culture No. 201 sk. Stained smear from Sabouraud's media; four days' growth. Showing the typical spherical, stained or unstained cells and also a few mycelial articles.

stained, visible as a highly refractile body, but at one pole of the cell, in contact with the nucleus or partly covering it, we find one or two faintly staining, pale red masses, varying very considerably in size and outline; sometimes as large as the nucleus or again very much smaller; sometimes round or spherical, again irregular or somewhat triangular. It appears probable that these represent the vacuoles of the cells.

We found also in a number of the specimens, in either hanging drops or smears, a varying number, usually few, of very large, spherical cells which take none of the stains and which probably are the so-called "Dauerformen" or durable cells.

In these organisms (No. 201 sk., No. 203 sk. and No. 203 st.) then we find usually spherical forms, a few elliptical forms and occasionally mycelial forms. The cells show a double contoured cell wall, and apparently contain a nucleus or several nuclei, one or more vacuoles, one or two dark motile granules, and possibly a varying amount of granular material. We have never been able to demonstrate spores in any of these organisms, nor have we seen spore sacs. About all that we can state from this study of the morphology of the organisms is that they are not to be considered as ascomycetes.

The cells of the other organism (No. 201 st.) differ from those of the three just described in that the majority are oval or elliptical rather than

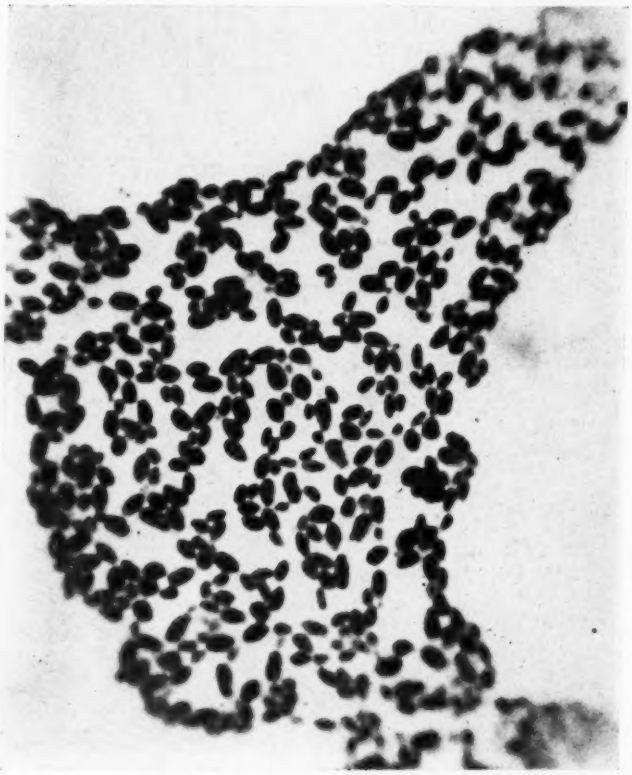

Fig. 7.—Culture No. 201 st. Stained smear from Sabouraud's media, four days' growth, showing the oval, deeply stained cells.

spherical, this difference being particularly marked in the younger cultures. If, however, we examine old cultures (four to eight months old) we find that most of the cells have the same form and appearance as those of the three strains first described.

The cells of No. 201 st. also stain more diffusely and more deeply with all stains (methylene-blue, thionin, fuchsin and hematoxylin) than do the others; the staining, however, is not even but is rather granular. There is considerable variation in the depth of the staining of individual cells. In some cells we see an unstained, rather refractile structure, usually spherical, which is in all likelihood the nucleus. Any other morphologic features are

masked by the staining of the granular matter within the cells. We find also in smears from these organisms mycelial forms such as were noted in the other organisms. Again no ascospores have been seen (Figs. 7 and 8).

Examined in the hanging drop, whether suspended in broth or in neutral red solution, the appearances of the structures within the cells of No. 201 st. are the same as in the other three organisms. The only thing then that differentiates culture No. 201 st. from No. 201 sk., No. 203 sk. and No. 203 st. is the cell outline and the reaction to stains in the earlier periods of growth. A small number of oval, granular staining forms, however, are seen in the young cultures of the three latter organisms, similar to the forms

Fig. 8.—Culture No. 201 st. Stained smear from Sabouraud's media, four weeks' growth, showing spherical forms, some unstained, some irregularly stained.

seen in No. 201 st., and furthermore in old cultures of No. 201 st., many of the cells assume the spherical forms of the other three; it is therefore difficult to separate these four organisms on a morphologic basis and it is possible that different rates of growth or a tendency to adhere to a particular form, representing possibly a stage of the life cycle of the organism, may account for the morphologic differences noted above.

The cultures of the four organisms on Sabouraud's media (using Difco peptone and Difco Standardized Maltose) are all very much alike, excepting that again the strain isolated from the stool of patient F. E. differed at times from the other three. The usual growth was profuse, white, and raised,

the edges were rather sharp, and the cultures showed only a slight tendency to spread over the surface of the media. The surface of the growth shows slight irregularities but no distinct wrinkling; it was glistening and appeared moist. No development suggesting aerial hyphae was noted. At times outgrowths from the streak were seen on the upper portion of the slant, where drying had begun; these were linear and usually rather short and could be noted with the naked eye as fuzziness at the edge of the growth. The appearance of these outgrowths was, however, by no means regular or constant in any of the four strains.

Fig. 9.—Microphotograph of culture No. 203 st., grown for seven days on Sabouraud's media plate, showing the typical mycelial outgrowth of the monilia and the grouped masses of conidia scattered along the mycelia. The entire field is shown in order to demonstrate the various planes in which the mycelia grow out, since they grow in the media and not on the surface.

At times the growth of No. 201 st. differed from the above description. The growth on the Sabouraud's media was not profuse nor yet scanty but considerably less than in strains No. 201 sk., No. 203 st., and No. 203 sk.; the growth was raised and colorless; at first translucent, later opaque. With the exception of these differences, the growth of No. 201 st. corresponded generally to the description of the other three given above. This particular strain developed, with time and after continued transplantation, a growth similar to or identical with the white, glistening opaque type which tended to replace the colorless translucent type. Possibly again this difference in

the appearance of the culture, like the difference in the appearance of the cell forms, was due principally to the varying rates of growth.

All the organisms grew well in a maltose-peptone-water media; both of the strains from patient I. S. (No. 203 st. and No. 203 sk.) showed a tendency toward bottom growth, no pellicle being formed; both of the strains from patient F. E. (No. 201 st. and No. 201 sk.) were top growers and formed a pellicle or thin scum which followed the fluid up the wall of the tube when the latter was wetted with the media. Our experience, however, with observations concerning top and bottom growth of organisms of this type, is such

Fig. 10.—Microphotograph of culture No. 201 sk. on Sabouraud's media plate, seven days old, showing the mycelium connecting the grouped masses of conidia which are typical of the monilia.

that we are inclined not to lay too great stress upon this matter as a factor in differentiating these organisms.

Observations regarding morphology and the gross cultural characteristics of these organisms have given us but little aid in placing or identifying them biologically. The form of growth, their mode of reproduction is the only criterion to aid us in this direction. We have made cultures of the four organisms in thin pour plates, using the Sabouraud's agar medium. The plates were placed in a moist chamber at 37° C. In some cases the typical outgrowth occurred within forty-eight hours, again not before a week or ten days; in some cases all colonies showed the outgrowths, in others only a

single or a few scattered colonies showed the typical forms. At times none of the colonies showed the mycelial outgrowth, and only the yeast-like forms were present. In all four organisms at some time or another, however, one or more colonies showed the typical outgrowth described below.

The typical mycelial outgrowths are evident, first, as a few elliptical cells pushing out from the mass of the colony which otherwise is composed of round or elliptical yeast-like cells. One or several round or elliptical cells appear at the free end of this first cell and this process of additional cells appearing at the end of a cell may continue, and so a mycelium of varying length is produced. The length and thickness of these mycelial cells is quite

Fig. 11.—Microphotograph of culture No. 201 sk. on Sabouraud's media, seven days old, under high magnification, showing the typical grouped masses of conidia and an extension of the mycelium composed of rather short oval cells, at the junction of two of which we find at places smaller, more spherical cells (conidia) which by budding in situ may later form masses similar to those shown lower down on the mycelium.

variable. They are not constantly elliptical but may be rectangular, with great exaggeration of the length; the width may be only half that of the usual oval or spherical cells. Occasionally they show branching, but this is not frequently noted. At times these mycelia are long—several times the length of the colony, or again they may be short, composed only of two or three cells. At the junction of these mycelial cells, usually, but not constantly, several round or oval cells appear forming rosettes. Usually the rosettes of cells increase by budding until we have at the junction points a mass of iregularly rounded or oval cells, giving the typical growth form. At

times these hyphae are thrust out into the media without the appearance of any rosette of cells at the junction points and in some cases the more typical form appears later (Figs. 9, 10, 11, 12).

At no times have we noted aerial hyphae, asci, spore sacs or any type or endospores. We have noted only the conidia (exospores) which form the mass of the growth.

Since no perfect forms of fructification are developed, these organisms, at least for the present, belong among the Fungi imperfecti, and according to the classification of Lindau in Engler-Prantl,[18] among the hyphomycetes. Further consideration of these classifications would place these organisms as monilia.

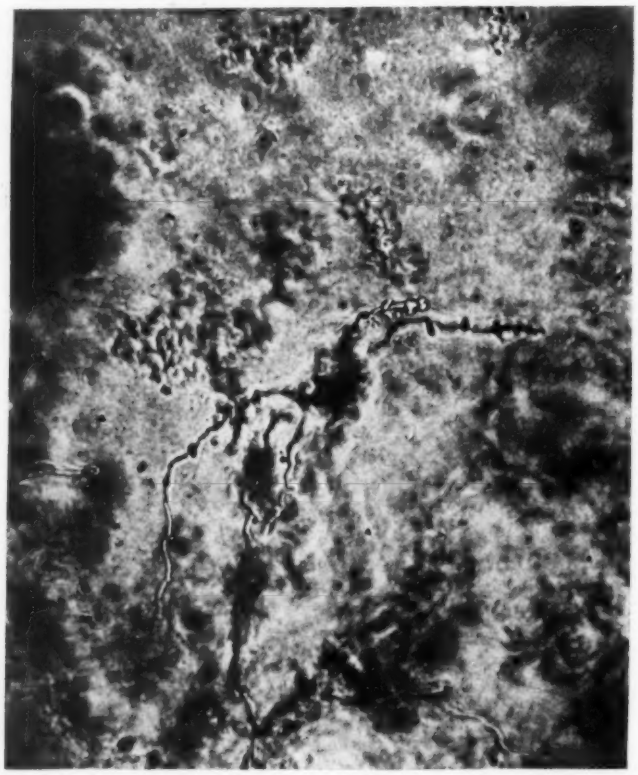

Fig. 12.—Microphotograph of culture No. 203 sk. on Sabouraud's media plate, seven days old, showing the simpler mycelial outgrowth with only a few conidia at the junction of two cells. These forms may later develop into the forms shown in Figs. 9 and 10.

Castellani and Chalmers[19] have attempted to classify the species of monilia on the basis of their action upon various carbohydrates and have, on this basis, differentiated a large number of species. The experience of one of us with a large number of strains of monilia (in as yet unreported work) and the experience of others makes it appear that this procedure is not satisfactory as a method for species differentiation. Castellani has himself noted that the monilia tend to lose with time (sometimes rapidly, sometimes more slowly) their power to ferment certain carbohydrates. We shall, therefore, not attempt to use the classification as outlined by Castellani and

Chalmers, but shall simply place these organisms in the genus monilia (Persoon).

We have, nevertheless, studied the actions of these four organisms on various carbohydrates, upon gelatin and milk, and Table I gives the results of this study. We wished principally to determine whether the two organisms isolated from the skin and feces of each case were identical or not.

It appears from this series of sixteen carbohydrates that the organisms isolated from the two patients are not identical organisms. The organisms from patient I. S. are distinctly more active in attacking the carbohydrates and in gas production than the organisms from patient F. E.

If we compare the actions upon carbohydrates of No. 201 sk. and No. 201 st., we find that these two organisms show the same actions upon all carbohydrates except upon xylose, melezitose and trehalose, and here the activity in fermentation varies irregularly. In comparing No. 203 sk. and No. 203 st. we find that these two differ only in their action upon xylose. We are inclined to believe that the differences noted between the two cultures from patient F. E. and the two from patient I. S. are negligible. Similar variations in the action upon the different carbohydrates have been noted a number of times in individual strains of monilia, when these were tested in the carbohydrates at various times after isolation.

We are, as a result of studies carried on in this laboratory, inclined to believe that the reactions of the monilia upon dextrose, levulose, maltose, saccharose and lactose are fairly constant and fixed qualities of the biologic activity of the various monilia; possibly the action upon inulin and dextrin is also quite definite. If the fermentation of carbohydrates is then to be used for purposes of differentiating monilia, we believe the first group mentioned above should be the only one relied upon for this purpose.

If we follow this scheme, it appears that the two organisms isolated from the skin and feces of patient F. E. are in all likelihood identical and the two isolated from patient I. S. are also probably identical, but the organisms from the two cases are apparently not identical.

Our studies then demonstrate the following facts: first, that monilia may be the causative agent of so-called Erosio interdigitalis; and second, that the organisms which appear in the dermatologic lesions may also be found in the feces of the affected individual.

DISCUSSION

The significance of the finding of the identical organism in the skin lesions and in the feces of these two cases may admit of two interpretations—either the occurrence and development of the organisms in the intestinal canal may have antedated the development of the skin lesions and the latter may be secondary to the intestinal infection, or the intestinal infection may have followed the dermatologic infection, and be due only to swallowing of the organisms from the hands, or swallowing materials contaminated through being handled by the patient; in which case we would consider the intestinal infection as simply chance and of little or no significance. It is, of course, impossible to state which of these explanations is correct, but it appears prob-

TABLE I

	DEXTROSE	LEVULOSE	MALTOSE	GALACTOSE	SACCHAROSE	INULIN	LACTOSE	DEXTRIN	ARABINOSE	XYLOSE	RHAMNOSE	RAFFINOSE	MANNITE	SALICIN	MELEZITOSE	TREHALOSE	GELATIN	MILK
F. E. Skin 201	AG	AG	AG	O	O	O	O	A	A	AG	O	O	O	O	A	AG	+	ALK cg
F. E. Stool 201	AG	AG	AG	O	O	O	O	A	A	O	O	O	O	O	O	O	+	ALK cg
I. S. Skin 203	AG	AG	AG	A	AG	O	O	AG	O	O	O	AG	AG	O	O	AG	+	ALK cg
I. S. Stool 203	AG	AG	AG	A	AG	O	O	AG	O	A	O	AG	AG	O	O	AG	+	ALK cg

A = acid production; G = gas production; O = neither acid nor gas production;
+ = growth along the line of stab but no arborescence; ALK = production of alkaline or no change of reaction; cg = coagulation of casein but no digestion (a soft coagulum is formed).

able, in view of the absence of general lesions and the absence of general effects in this type of infection, that the intestinal lodgment of the monilia is secondary to an initial infection of the skin.

If we consider our own findings and the findings of others concerning the organisms causing Erosio interdigitalis, it appears that in no case have blastomyces been demonstrated as the causative agents, since in no cases have cultures been obtained which show aerial hyphae. It is apparent, however, that ascomycetes have been demonstrated in some cases (by Hudelo, Sartory and Montlaur and Greenbaum and Klauder) and that Fungi imperfecti have been demonstrated in others—specifically endomyces, or botrytis and monilia,* these latter representing all rather closely related or similar organisms. It appears also that some of the unidentified organisms described by various observers may well be placed as either ascomycetes or as monilia, judging from the incomplete description given of the organism or the cultures obtained. With the present evidence then it appears that Erosio interdigitalis may be caused by either ascomycetes or Fungi imperfecti, especially members of the oosporae or the botrytidae.

It would appear further that the various names which have been applied to this type of dermatologic infection are not satisfactory or justified, in view of the types and the variety of etiologic agents, since they all carry the implication of either blastomyces or saccharomyces as the causative agents. If it is essential that the etiology be referred to, a term implying simply that fungi act as the causative agents would be justified.

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*Recently we have isolated a monilia from a third case of Erosio interdigitalis; this was an early case. It is questionable whether this third organism is identical with either of the two reported in this article.

CALCIUM DISTRIBUTION IN BLOOD*

By Paul L. Kirk and C. G. King, Pittsburgh, Pa.

IT HAS long been recognized by the medical profession that sufferers from various types of jaundice often show a delayed blood coagulation time. This in many instances proves fatal following operations. Walters[1] reported that during the years 1918, 1919 and 1920, over 50 per cent of all patients operated upon with obstructive jaundice at the Mayo Clinic, died from intra-abdominal hemorrhage, or on necropsy, showed large amounts of blood in the intraperitoneal cavity. Analogous cases without jaundice during the same period, showed only 6 per cent fatalities from intraabdominal hemorrhage. This effect is presumably due to or parallel with, the presence of some biliary constituents in the blood. Lee and Vincent,[2] and Walters,[3] have shown that the intravenous injection of calcium chloride or lactate shortens the coagulation time, and lowers the proportion of fatalities following operation. Such a procedure is now widely used by physicians, but does not give uniformly satisfactory results.

It has been pointed out by Neuhausen and Pincus[4] that the inorganic constituents of the blood may be present in three forms: ions, undissociated molecules in equilibrium with the ions, and nonionizable compounds with some of the organic constituents. They have also shown that all the common inorganic constituents of pig serum are totally diffusible, with the exception of calcium. The nondiffusibility of part of the blood calcium has long been recognized, and variously reported as from 25 per cent to 50 per cent nondiffusible.

The methods used in studying the calcium distribution in blood have been divided into four general classes: (a) An attempt has been made to determine the concentration of the calcium ions[5] by electrometric methods, in which the value found was 20 per cent to 25 per cent. The method was unsatisfactory, and was stated to involve a possible error of ± 25 per cent of the value found. (b) Dialysis has been used by various investigators. Clark[6] and later Loeb[7] used simple dialysis. Loeb's work showed that all the serum calcium was dialyzable in the presence of large quantities of salt solution. This would indicate that the colloidal complex may break down to yield diffusible calcium, if dialysis is carried out over a considerable length of time. Against distilled water his results were roughly in agreement with those of other investigators, as to the percentage of diffusible calcium. The development of compensation dialysis by Michaelis,[8] Rona,[9] and their stu-

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dents, and later used by von Meysenbug and his students,[10] is the only important advance made in this method. Von Meysenbug and his students found from 20 per cent to 40 per cent of the calcium to be nondiffusible, by his method of compensation dialysis. This work was carried out with extreme care, but all of the investigations that were made by this method are open to the criticism that a Donnan membrane equilibrium is established, and does not allow free dialysis of the inorganic constituents of the blood. Bayliss[11] has most clearly demonstrated the existence of such a permanent equilibrium. (c) Various forms of ultrafiltration through semipermeable membranes have been used. Cushny[12] made use of collodion sacs. Richter-Quittner[13] used a Zsigmondy-Bachmann filter. Later, Marshall and Vickers[14] used collodion sacs, and Neuhausen and Pincus[4] adopted their method. The quantity of serum used was comparatively large, about 30 c.c., and the outsides of the filters were not immersed in water. Moritz[15] objects to this procedure for small quantities of serum, because of the drying of the membrane, and the deposit of a film of serum on the filter. He used small collodion sacs, immersed in distilled water, and under a negative pressure not to exceed 180 mm. of mercury. (d) A method of determining that portion of the blood calcium which is active in producing the clot, based on the clotting time, has been used by Bauer and Narnickol,[16] and Vines.[17] The method consists essentially of the comparison of the clotting time of two portions of oxalated or citrated plasma, to one of which is added a calcium chloride solution, and to the other a portion of the blood being studied. Such a procedure gives little if any information bearing directly on the state of the blood calcium, since the assumption is made that the only factor involved is the calcium of the added blood.

Cameron and Moorhouse[18] have recently reported a study of the calcium distribution between dog's blood and the cerebrospinal fluid, and assumed that the total calcium content of the latter was the same as the diffusible calcium of the blood. The average value given on this basis was 53 per cent diffusible.

EXPERIMENTAL

A. *Methods.*—Of the four methods previously mentioned for the estimation of diffusible calcium in blood, the method of ultrafiltration under low pressures, seemed to be the least open to criticism, and was adopted for this investigation.

Most of the low pressure ultrafiltration has been done using negative pressure. Because of the high loss from evaporation of the filtrate, using this method, the use of positive pressure was adopted. The pressure employed approximated 150 mm. of mercury at all times. It was never allowed to go above 180 mm. or below 120 mm. of mercury. It has been pointed out by Neuhausen and Pincus[4] that between these pressures, there is no change in the character of the filtrate, and by previous investigators, that there is no precipitation of protein. It is also sufficient to induce a fair rate of filtration. The objections of Moritz, previously mentioned, were overcome in this method of ultrafiltration by filtering into an empty vessel which was practically

closed to the air by a loose joint, and which completely enclosed the collodion sac. We had no difficulty from drying of the filter, and no film was deposited on our filters except when exceptionally long periods of filtration were necessary. Our evaporation loss was checked for a period of ten hours, and was found to be about 0.02 to 0.03 c.c., which was considered negligible. We believe this technic to be an improvement over the methods described in the literature, because of its simplicity and dependability. Once started, our filters required no attention until filtration was complete. There was no variation in pressure, and the simplicity of our apparatus was the equal of any yet described.

Past investigations on blood by ultrafiltration have, in practically every case, been conducted on serum. Since this study was essentially concerned with the factors involved in coagulation, it was considered preferable to use plasma, because it had undergone no changes from coagulation. The difficulty encountered here was in obtaining an anticoagulant which did not affect the calcium. Hirudin, or the extract of the medicinal leech, was decided on, as being the most suitable anticoagulant, following the suggestion of Richter-Quittner.[19] It has been shown that this material acts as an antithrombin, and has no action on the mineral constituents, whereas, oxalates, citrates and fluorides either precipitate or bind the calcium.[20, 21, 22] It has likewise been shown that these materials affect the P_H of the blood.[23] Some difficulty was encountered in obtaining this extract, and the first portion of this study was consequently made on serum. Later, leeches were purchased, and extracted in our own laboratory. This procedure was not entirely satisfactory, due to the difficulty of standardization of the extract, and its tendency to undergo rapid bacterial decomposition. The material was repeatedly pasteurized, and kept in the ice box. Its action was satisfactory enough of the time to give significant results, however.

The blood calcium was determined by the method of Kramer and Howland.[24] This method, while somewhat more laborious, gives more satisfactory results than the methods of Lyman,[25] or of Kramer and Tisdale.[26] An analysis was made on the plasma or serum of the original sample. A duplicate analysis was made on the filtrate, after the ultrafiltration. All analyses were made in an identical manner, so that the results are comparable, even though they may not represent absolute values. Checks were run whenever the size of the sample permitted it.

All ultrafiltration, and handling of the blood previous to ultrafiltration, was carried out as nearly as possible at 37° C. For this purpose, samples were received in warm tubes, carried in small Dewar flasks, centrifuged in warm cups, and ultrafiltered in an incubator at 37° C. immediately. Our experience indicates that it is preferable to work at lower temperatures. A very few hours at this temperature is sufficient to induce vigorous bacterial growth in case of accidental contamination. Moreover, it has been shown by Moritz[15] that there is a progressive increase in the percentage of diffusible calcium, in blood standing at room temperature.

No check on the P_H of the blood was made. It has been shown by Menten,[23] however, that hirudin added to blood does not affect to any extent the hydro-

gen-ion concentration. In general, no other treatment was used which would affect the P_H, and the blood was not allowed to stand for long periods of time.

In practically every case the ultrafiltrates were tested for protein, either by the biuret reaction, or by the xanthoproteic test, or both, and in no case was a positive protein test obtained. All jaundiced blood was tested for the presence of bilirubin, by the method described by Kapsinow,[27] using Obermayer reagent. The results were entirely satisfactory. Many of the ultrafiltrates were similarly tested, and no positive tests were given. After filtration, the deep color of the filters also showed the retention of bile pigment.

B. *Apparatus.*—The ultrafilters consisted of collodion sacs, fastened to test tubes, the bottoms of which had been removed. The collodion was made from a high viscosity nitrocellulose, 10 g. of which was dissolved in a mixture of 50 c.c. absolute ether, 50 c.c. absolute alcohol, and 10 c.c. glacial acetic acid. Olive oil was substituted for the acetic acid in preliminary experiments, following the suggestion of Moritz,[15] but the resulting sacs were not as strong as those made with the acetic acid. The use of glacial acetic acid in this manner has been recommended by Eggerth.[28] The sacs were made by filling tubes with collodion, centrifuging for two minutes and draining. This was repeated a second time. After the second draining, the tubes were dried with constant rotation for ten minutes. They were then immersed in water and the sacs could be easily removed in about ten minutes, by holding them under water and loosening them in the tubes. The tubes were of such size that the sacs fit tightly on the outside of test tubes. The bottoms were cut from the test tubes, the sacs slipped on, and bound there by a firm wrapping with silk thread. No difficulty was experienced from leakage or loosening of the sac.

Large test tubes were melted down at the open end, to such a size that the filter tube would just enter, but so that the flare on top of the filter tube would fit snugly. These large tubes served as receiving vessels for the filtrate.

The apparatus used for application of pressure to the ultrafilters, consisted of a five gallon carboy to which suitable connections were made with pressure lines. The carboy was stoppered with a three-hole rubber stopper, which was wired in tightly, and covered with sealing wax. Through one hole was placed a tube connected with a stopcock on the water line. This tube extended to the bottom of the carboy, and served as a siphon for emptying the vessel. Through a second hole was placed an open manometer, calibrated for 120 mm., 150 mm., and 180 mm. Through the third hole was connected a line to the ultrafilters in the incubator. In this line was included a three-way stopcock, one branch of which opened to the air, to allow release of pressure, and also the intake of air on siphoning out the water from the carboys. The end of this line was branched, with a stopcock on each branch and the ends of the branches were fitted with small, flexible, rubber stoppers, which exactly fit the ultrafilter tubes. All lines were constructed

entirely of glass and pressure tubing, with as little of the rubber tubing as was practical. All joints in the apparatus were wired, and trebly shellacked. No difficulty with leakage was encountered. The pressure was obtained by displacement of air in the carboy by water.

The apparatus used for the calcium determinations followed closely the description in the original article of Kramer and Howland.[24] The titrations were made entirely with 10 c.c. burettes, calibrated in twentieths of a cubic centimeter. For ashing the blood, both platinum crucibles and Vitreosil crucibles were used. No previous record has been made of the use of Vitreosil for this purpose. Some difficulty was experienced with the Vitreosil. The results seemed to run lower in general than in platinum, on the same samples. A deposit of crust was formed by the ash in the Vitreosil crucibles, which did not readily respond to treatment with dilute acids, so that these crucibles were replaced entirely with platinum. All ignitions were made over Meker burners.

C. *Experimental Data.*—

1. *Normal Blood.* Blood was taken in most cases from hospital patients whose disorders were such that they would not be expected to affect the calcium distribution. A few were taken from patients in which the calcium was found to be definitely affected. These will be included under normal blood, signifying merely that they showed no jaundice. The concentration of calcium found is in every case expressed in milligrams per 100 c.c. of plasma or serum.

CASE 1.—Middle-aged, colored, male, with gastric disorder. Blood slightly clotted during filtration.

Total Ca, 8.49; ultrafilterable Ca, 67.4 per cent.

CASE 2.—Middle-aged male with some hypertension. Blood clotted slightly during filtration.

Total Ca, 7.58; ultrafilterable Ca, 67.7 per cent.

CASE 3.—Middle-aged male with gastric disorder. The sample was small which greatly increased the possibility of error.

Total Ca, 9.24; ultrafilterable Ca, 80.9 per cent.

CASE 4.—Male, age twenty-three. No abnormal conditions.

Total Ca, 7.43; ultrafilterable Ca, 72.3 per cent.

CASE 5.—Female, early middle age, a short time after induced abortion.

Total Ca, 7.69; ultrafilterable Ca, 56.0 per cent.

This low value can be correlated with the effect of pregnancy, as will be indicated by the next two cases.

CASE 6.—Female, about twenty-five years of age, in late pregnancy. Filtration made on serum.

Total Ca, 12.51; ultrafilterable Ca, 26.85 per cent.

CASE 7.—Middle-aged female in late pregnancy.

Total Ca, 13.94; ultrafilterable Ca (serum), 16.87 per cent.

2. *Jaundiced Blood.* Some of the samples of jaundiced blood were allowed to clot, and serum runs made. Others clotted due to insufficient hirudin, while some were made on plasma.

CASE 1.—Middle-aged colored man, slightly anemic, jaundiced, due to cirrhosis of the liver, caused by acute syphilis. Treatment was being given in the form of neoarsphenamine injections. Icterus index was 50 to 60.

Total Ca, 9.80; ultrafilterable Ca, 45.95 per cent.

CASE 2.—Female, late middle age, slightly jaundiced. Cholelithotomy performed one month previously. The original icterus index was 20. At the time of drawing sample it was 10.7. The patient was recovering from marked anemia.

Total Ca, 11.39; ultrafilterable Ca (serum), 55.1 per cent.

CASE 3.—Female, middle age, very slightly jaundiced from obstruction of the common bile duct.

Total Ca, 7.86; ultrafilterable Ca, 66.5 per cent.

CASE 4.—Female, seventy-two years of age, obstructive jaundice, highly developed.

Total Ca, 4.31; ultrafilterable Ca (serum), 50.6 per cent.

CASE 5.—Male, late middle age, slightly anemic, slightly jaundiced due to obstruction. Clotting time, 10+ minutes.

Total Ca, 6.94; ultrafilterable Ca (serum), 59.9 per cent.

CASE 6.—Female, late middle age, previously jaundiced four times. Not jaundiced at time of drawing sample. Diagnosed as having gallstones.

Total Ca, 7.91; ultrafilterable Ca, 64 per cent.

CASE 7.—Female, middle age, highly jaundiced due to obstruction. Coagulation time, 6.5 minutes.

Total Ca, 8.52; ultrafilterable Ca (serum), 48.2 per cent.

3. *Other Pathologic Conditions.* One case only of advanced nephritis and uremia was obtained.

CASE 1.—Male, late middle age, advanced nephritis and uremia. Blood clotted somewhat due to insufficiency of hirudin.

Total Ca, 7.49; ultrafilterable Ca, 51.15 per cent.

4. *Anticoagulants.* Dog's blood was used in this study. All samples were drawn at the same time from an artery, while the animal was anesthetized by ether. The anticoagulants used were hirudin, sodium citrate, potassium oxalate, and sodium fluoride. The total calcium of the blood plasma was 8.68 mg. per 100 c.c.

ULTRAFILTERABLE CALCIUM

	Hirudin	Sodium citrate	Potassium oxalate	Sodium fluoride
First Series	72.7 per cent	86.2 per cent	0 per cent	13.98 per cent
Second Series	69.7 per cent	100.0 per cent	0 per cent	0. per cent

The second series of samples were kept for twenty-four hours in the ice box. The second sample treated with hirudin clotted, and the serum was filtered. The first series was run immediately after drawing.

5. *Biliary Constituents.* The only biliary constituents which were tested for their effect on the blood calcium distribution were sodium taurocholate and sodium glycocholate. These were mixed in equal portions, and 0.26 g. was added to 20 c.c. of normal human blood, treated with hirudin. This may have affected the P_H of the blood to some extent.

Total Ca, 7.43; ultrafilterable Ca, with bile salts, 55.2 per cent; ultrafilterable Ca, normal, 72.3 per cent.

D. *Discussion.*—Since only a limited number of jaundice cases were available during the period of this investigation, and since considerable difficulty was encountered in obtaining blood which could be considered as normal in respect to the calcium distribution, it is considered dangerous to make too sweeping generalizations. It appears, however, that the percentage of diffusible calcium is somewhat lower in jaundiced than in normal blood, and this lowering is roughly proportional to the severity of the jaundice. Since there are several other conditions in which the percentage of diffusible calcium is considerably lower than in the normal adult, without any marked decrease in the coagulability of the blood, it appears probable that the lowering of the diffusible calcium in jaundice is not the only factor involved in increasing the coagulation time, if it is involved at all. Schultz[29] has shown that platelet deficiency is a factor in the diminished coagulability of the blood in anaphylaxis, and we plan to investigate this factor in jaundice in the near future.

Another factor which has not been sufficiently studied in regard to diffusible calcium, is the amount and kind of protein present in the blood in jaundice. Loeb and Nichols[30] have shown that the calcium is bound to certain types of blood proteins. Perhaps there are other types which do not bind calcium, and a disturbance in this protein balance might affect the degree of diffusibility of the calcium.

Normal Cases 6 and 7 indicate that pregnancy probably has a very considerable effect on the distribution of the blood calcium. Total calcium seems to be high and ultrafilterable calcium to be abnormally low. This does not agree with the results of Cameron and Moorhouse.[18]

A comparison of the results obtained upon plasma and serum indicate that very little if any difference may be expected so far as the ultrafilterable calcium is concerned.

It was found that the common anticoagulants should not be used in studies on blood calcium distribution. Oxalate eliminates all the ultrafilterable calcium in a short time. Fluoride is slower in action, but on standing, puts all the calcium in a nondiffusible form. Citrate, however, slowly increases the diffusible calcium. Probably the removal of the calcium ions to form a citrate-calcium complex displaces the equilibrium between the ionic and colloidal calcium, with removal of ions and further formation of the diffusible citrate-calcium complex. This action is rather slow, as is also the action in long dialysis of blood against salt solution,[7] indicating that the calcium equilibrium is only very slowly displaced.

SUMMARY

The total and ultrafilterable calcium has been estimated in the blood of seven persons showing evidence of a jaundiced condition.

Comparison with normal cases indicates that the percentage of ultrafilterable calcium found in this condition is below normal. The difference is not thought to be sufficiently great, however, to account for the frequent delayed coagulation.

Preliminary indication is given that the ultrafilterable calcium is low during pregnancy.

The use of citrate, oxalate, and fluoride as anticoagulants were found to cause a very marked change in the calcium distribution in blood.

An improvement in the procedure for studying the ultrafilterable constituents in blood has been developed.

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CYTOMORPHOSIS OF THE TUBERCLE BACILLUS AND OTHER ACID-FAST MICROORGANISMS*

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THE tubercle bacillus, on account of its economic importance as a disease-producing microorganism, has been studied probably as exhaustively as any one other low form of life, and yet, from a practical standpoint, there are still many logical questions which arise involving the bacillus itself which have only been touched upon superficially and inconclusively. In explanation of this may be taken the slow growth of this microorganism and its polymorphic nature both in the culture tube and in the body. That there is a relation between the morphology and the character has been suggested a number of times, but all efforts to correlate the two, especially in the human or animal economy, has been of no avail. This assumes importance, especially when it is realized that our present day clinical determinatives of the activity of tuberculosis take into consideration only the body tissue or cellular reaction for the purpose of interpretation, and the bacillary factor is dispensed with in the simple terms negative or positive. Thus also our experimental efforts leave much to be desired from the standpoint of bacillary interpretation as to the effect and value of certain therapeutic attempts. Irregularity in length, thickness, contour, and form (as well as in mode of division) is the characteristic of the tubercle bacilli and other acid-fast microorganisms, according to Miehe,[1] who studied the morphology and systematic classification of the tubercle bacillus. The tubercle bacillus according to him is an amycelial mold like yeast, the unit being the cell and not the mycelium. It belongs to the family mycobacteriaceae and is not a degenerated parasitic form of the saprophytic molds found in nature, as suggested by Coppen-Jones,[2] Bruns and others.

In scanning the literature, and after having examined numerous microscopic specimens, both from cultures of tubercle bacilli and from animal or human tuberculosis, one is impressed with certain striking morphologic characteristics which merit consideration in following the consecutive changes undergone by the tubercle bacillus in the culture tube or in the body. The long filamentous and branching types have excited intense interest, especially among investigators interested in the classification of this microorganism. The majority of reports record these forms especially in avian cultures, and in mammalian cultures grown under abnormal conditions. They have also been recorded as occurring in certain positive sputums.[3] Intermediate to these long filamentous branching forms are the usual longer and shorter straight and curved forms commonly occurring in the average well-grown

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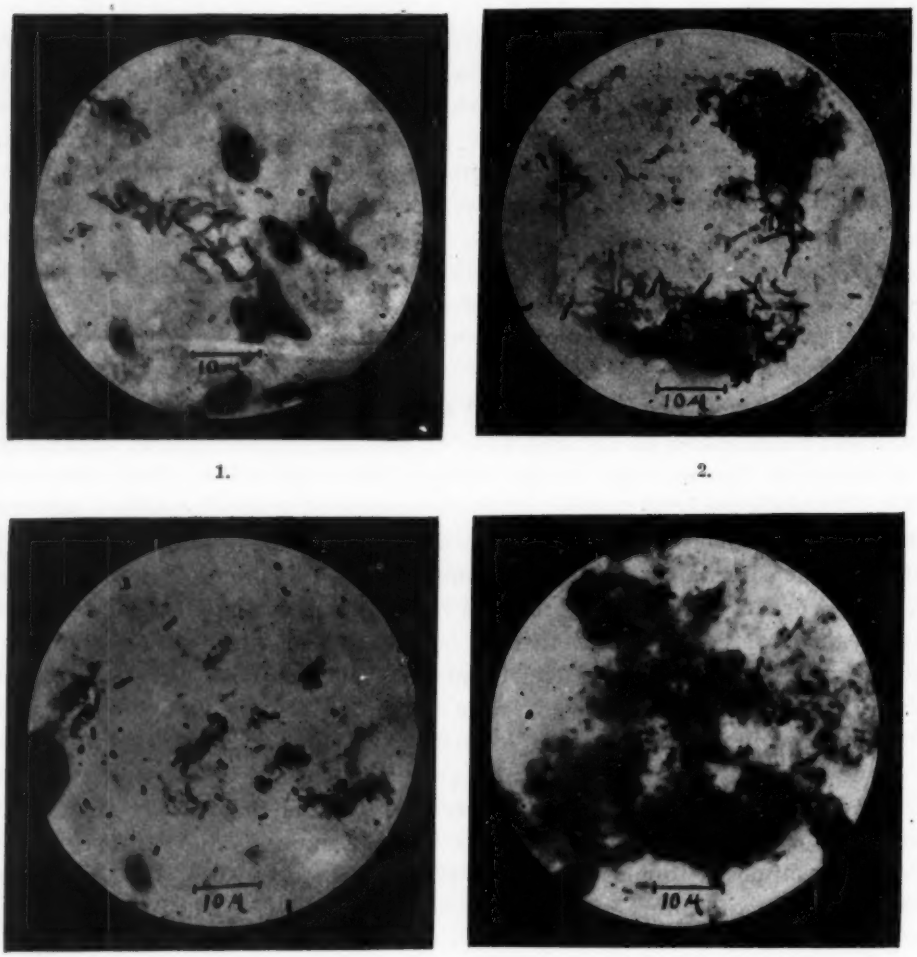
culture and seen in the ordinary sputum from open cases of pulmonary tuberculosis.

In 1905 Carl Spengler[4] emphasized a smaller acid-fast form which he termed "Splitter" and which has led to a great deal of discussion and probably had much to do with the identification of Much's granules. Practically neither the "Splitter" nor "Much Gram granules" have proved of value[5] but the "Splitter" are of interest here because of their specific acid-fast character. Spengler utilized only groups of "Splitter" for diagnostic purposes and describes them as being acid-fast clusters or granules. He considered them involution forms of the tubercle bacillus of reduced vitality and virulence. Their resistance to chemicals is less than that of the ordinary rod forms. They are found in cultures only where the nutrition is reduced and unfavorable to the bacilli. They occur as a rule with bovine bacilli when the nutrient medium is unfavorable and not so frequently with human bacilli. Whether these "Splitter" are in any way related to the more deeply-staining segments occurring normally in many of the bacilli is not elucidated.

In an ingenious experiment Levy[6] succeeded in growing human tubercle bacilli at temperatures of 41° and 42.5° C. but did not succeed by this means in altering the morphology of the cultures as compared to those grown at 37° C. In reviewing the subject of the existence of spores he points out that there exist in tubercle bacilli doubly refractile bodies which take a deep, dark-red fuchsin stain and resist decolorizing with mineral acids. If these are spores or "resting bodies" they do not conform to the classical definition with regard to the resistance to heat, etc. Szepesi,[7] after staining tubercle bacilli by means of carbolfuchsin, impregnated them with silver and found that the bacilli did not uniformly take up the silver nitrate but black granules appeared in certain locations, either at the ends or in the middle as 1, 2 or 3 (never more) sharply marked off granules. It is believed that the granules consist of a combination of silver and protein, since they dissolve in potassium cyanide solution. They are not the same as the Gram granules which are supposed to consist of neutral fats, according to Much. The silver granules seem to differ only slightly from the polar bodies seen in Ziehl preparations and whether they are spores or not remains undetermined. Similar structures were not demonstrable in staphylococci, typhoid and paratyphoid bacilli.

Probably the most exhaustive and elaborate study of the changes occurring in human and bovine tubercle bacilli in culture is that reported by Wolbach and Ernst.[8] They studied four human and seven bovine strains of tubercle bacilli at various intervals during cultivation up to three to four weeks. In summarizing their results with the cultures of human and bovine origin, they state that during the first days of growth the form assumed by the tubercle bacillus is longer than that of the later stages, and these forms grow progressively shorter until the type form of the fully developed culture is reached. The irregularly stained and branched forms occur at a period of vigorous multiplication, and cannot be considered as involution or degenerate

forms. Neither the age of the culture used nor the medium on which it was grown affected the growth or microscopic appearances of the subcultures on egg. Likewise the reaction of the egg medium had no effect on the general characteristics and changes occurring at different ages. The changes described occurred with all the cultures used, though requiring different lengths



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Photomicrograph Series A.

Cytomorphosis of "Koch-Novy" tubercle bacilli grown on glycerol agar at 37° C.

1. Appearance of bacilli after incubation for one day. Acid-fast fuchsin-stained bacilli appear as black rods, nonacid-fast methylene-blue-stained bacilli appear as faint gray shadows.
2. Appearance of bacilli after incubation for one week.
3. Appearance of bacilli after incubation for one month.
4. Acid-fast fuchsin stained lipoid globules found after nineteen months at room temperature following one week at incubator temperature.

of time for their completion, varying from fourteen to twenty-six days. The greatest variations in form and staining reaction were found in rapidly growing cultures and they agree with Coppen-Jones that a favorable medium and free access of oxygen are best suited for the production of branched and filiform forms. The only interpretation of the great diversity of form assumed

by the tubercle bacillus when grown under most favorable conditions is that it is truly pleomorphic and should be classed among the higher bacteria.

It is only natural that the question of the changes occurring in tubercle bacilli in the culture tube and in the body should involve the problem of bacteriolysis, and Isabolinsky and Gitowitsch[9] recently report that the tubercle bacillus undergoes biologic changes when grown upon the liquid nutrient egg medium of Besredka which are viewed as bacteriolysis. After six weeks' incubation at 38° C. the number of Ziehl staining morphologically unchanged bacilli become fewer and the Much-Weiss forms increase. Under the influence of lipoid-containing substances, tubercle bacilli undergo all stages of lipolysis, from loss of acid-fastness to complete solution. The best results occurred with lecithin, olive oil and green soap. Sodium and potassium salts, as well as alkalies, possess no bacteriolytic properties.

The apparent hopelessness of utilizing the information on the tubercle bacillus at present at hand for clinical interpretation is voiced by Sorley,[10] who elaborately reviews the literature and reports the results of examination of sputums with a view to an interpretation of the morphology of the bacilli, the presence of "Splitter" and of Much granules. He concludes that "the presence of the granules implies simply that the bacillus is in a state of active reproduction, the presence of interpolar granules that the bacillus has attained a stage of greater or lesser maturity, and that, whether the bacilli be few or many, the severity of the disease and the prognosis are not necessarily affected thereby." Georgevitch[11] likewise sees no relation between the clinical evolution and the number of bacilli in the sputum or their morphology.

In 1923 Corper and Gauss[12] reported the results of a study of the viability of tubercle bacilli on culture mediums. It was found that fully grown cultures of human tubercle bacilli may remain viable on gentian-violet egg medium or 5 per cent glycerol-agar, whether kept in the ice box or in the incubator, for about four to eight months, so that successful transplants can be obtained on these mediums up to this time. Bovine tubercle bacilli are more resistant on these mediums and may remain viable (in the sense of furnishing subcultures) for from about eight to sixteen months (the latter in exceptional cases). The more vigorous and profuse growers seem to give successful transplants after longer intervals than the less vigorous. At that time it was also pointed out a culture may still contain sufficient viable bacilli to infect a highly susceptible animal like the guinea pig, when injected in large amounts, after nineteen months and still will not grow on artificial mediums (Theobald Smith).

In elaboration of these studies and on the basis of the information available as a result of the recent excellent contributions by Henrici[13] on bacterial cytomorphosis, it seemed advisable to study the changes that occur during the growth and loss of viability of the tubercle bacillus in the culture tube and if possible to correlate this with the morphologic changes noted in the animal body, giving careful attention to the length and staining properties of the tubercle bacilli. Since it is the acid-fastness that specifically characterizes the tubercle bacillus, none of the other nonacid-fast staining methods were utilized in

this preliminary study. Bacteriologists have most generally accepted the teachings that the morphologic characters of bacteria are invariable except for such pathologic changes as may be brought about by an unfavorable environment, with a minority believing that they exhibit a complex "life cycle" similar to that of protozoa or higher fungi, the various stages of which may be found only in varying mediums (Löhnis). The error in both these teachings is due to an insufficient consideration of the element of time and Henrici, as a result of quantitative studies of the morphologic characters of several species of bacteria, has arrived at a theory differing from either of the aforementioned. It is, briefly, that the cells of bacteria undergo a regular metamorphosis during the growth of a culture similar to the metamorphosis exhibited by the cells of a multicellular organism during its development, each species presenting three types of cells, a young form, an adult form, and a senescent form; and that these variations are dependent on the metabolic rate, the change from one type to another occurring at the points of inflection in the growth curve. The young or embryonic type is maintained during the period of accelerating growth, the adult form appears with the phase of negative acceleration, and the senescent cells develop at the beginning of the death phase. The term "involution form" implies changes associated with senescence and death. Increase in size of young growing cells may be six times that of resting cells and is accompanied by other changes in morphology such as disappearance of intracellular granules, the protoplasm becoming more hyaline and staining more deeply. Young cultures may also be more susceptible to harmful agents than are older ones, a parallel with multicellular organisms.

It is pointed out by Henrici[14] that he found the diphtheroid group as an exception, decreasing in size during the period of active growth in agreement with Clark and Ruehl's[15] findings. V. Cholera[16] with increasing rapid growth assumes a long, plump, relatively straight form; with decreasing growth the cells become more slender and curved (the typical "vibrio" form), and when growth is arrested and death begins the cells assume bizarre forms showing bulgings and "budding" and many become spherical. It is noteworthy that only the mature cells are spirella, the embryonic forms being bacilli and the senescent type cocci.

In order to obtain as much information as possible which might elucidate the problem of the changes occurring in the tubercle bacillus in the body, the studies to be recorded below were planned so that the morphologic changes occurring during the growth of not only tubercle bacilli but other closely related acid-fast organisms in the culture tube were studied. The more rapid growth and apparently larger size of the latter would lend itself better to more detailed microscopic examination as well as covering possible greater changes in morphology over a shorter time period. This phase of the study would also serve to note any differences between the more rapid growers and genuine tubercle bacilli, if such existed. The four strains of rapid growers studied were a Smegma bacillus, an Avian tubercle bacillus, an acid-fast organism "Day," and a rapidly growing avirulent strain of tubercle

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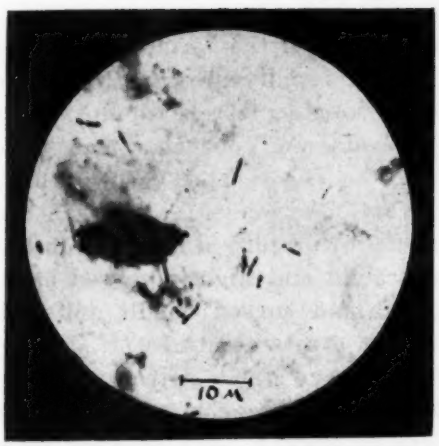

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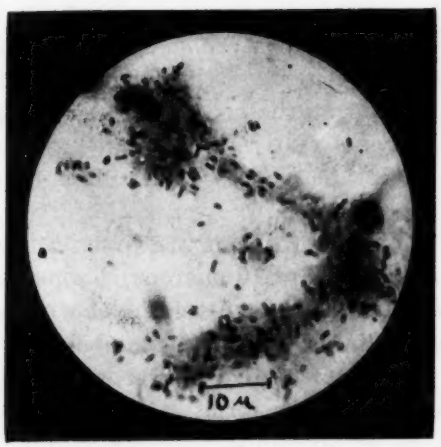

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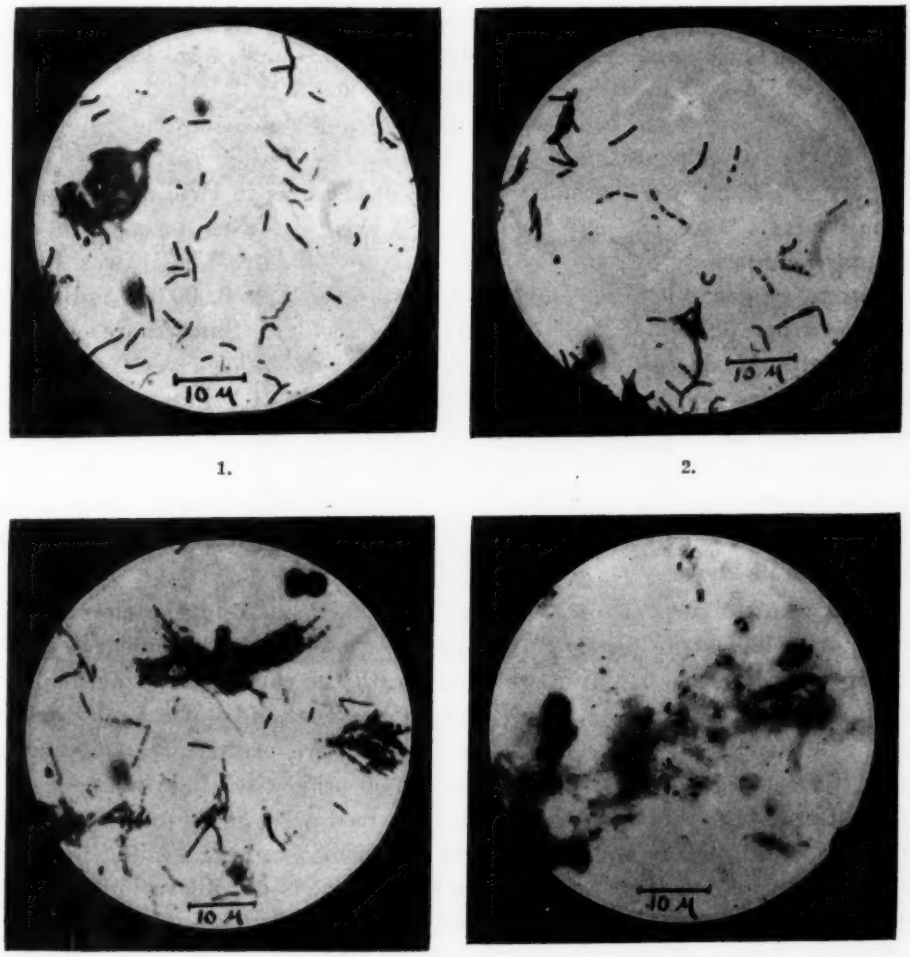
Photomicrograph Series B.

Cytomorphosis of "Day" tubercle bacilli grown on glycerol agar at 37° C.
1. Appearance of bacilli after incubation for three days.
2. Appearance of bacilli after incubation for one week.
3. Appearance of bacilli after incubation for one month.
4. Appearance of bacilli after incubation for two months.
5. Appearance of bacilli after incubation for four months.

bacilli ("Koch-Novy"). The latter was brought from Koch's laboratory in 1888 by Dr. F. G. Novy of the University of Michigan when it was still virulent,[17] while the former three strains were obtained from Dr. E. R. Long of the University of Chicago several years ago and have been maintained by weekly transfers onto new mediums. In our hands these strains gave profuse cultures in the incubator on glycerol-agar in one to two days. In this study no attempt was made to grow them on different mediums but young cultures one to two days old were transferred onto a series of 5 per cent glycerol-agar tubes which were then capped by paraffined cotton plugs and paraffined cloth to prevent drying out of the mediums. Smears were made from one of the tubes after incubation for one, two, three, and four days, one and two weeks, one, two, and four months, no tube being used twice for obtaining smears, thus avoiding the possibility of changes occurring incident to the introduction of a contaminator. The smears were stained after fixing, by means of steaming carbolfuchsin for thirty seconds, washed with water, decolorized with acid alcohol, and counterstained with methylene-blue for thirty seconds. Since it is almost impossible to obtain uniform direct smears from cultures, the bacilli were first ground up in a centrifuge tube and a uniform suspension made in saline solution to which there was added an equal volume of acetone and from which the smears were made. In general there was noted in the microscopic examination of the smears a marked variation in size and beading of the bacilli and only by a rather approximate quantitative examination of a large number of bacilli could deductions of any value be drawn. When, however, a series of slides were examined which had been taken at different intervals, there was a striking contrast between the size and shape of the bacilli as well as in the acid-fast beading or granules noted in the bacilli. In all the rapidly growing cultures the findings in this respect were practically uniform and they, therefore, can be reported in brief together. Minor individual variations occurred but, for the solution of the problem at hand, were of no especial significance. Of the variables, however, it was rather interesting that only one of the rapidly growing forms, the Koch-Novy strain, reputed to have originated from a tubercle bacillus, in the first days of cultivation revealed a large percentage of nonacid-fast bacilli on the glycerol-agar, by the staining technic used. This did not occur with the three other rapid growers. Uniformly, however, the four strains of acid-fast bacilli revealed during the first few days of growth quite a large number of long (up to 8 to 10 microns) filamentous straight and slightly curved bacilli interspersed with the usual shorter straight and curved bacilli uniformly fuchsin stained. At this time beading was to a great extent absent, although an occasional beaded and short bacillary form was seen. After one week's growth the long forms still persisted but beading, irregular and in streptococcus- or streptobacillus-like chains, began to appear. The striking forms at this time were the long strings of acid-fast beads in which the beads were a deep red while the intermediate parts were free from stain. The long forms of uniformly stained bacilli still were found at this period but the number was markedly decreased over the earlier periods. After one month the uni-

formly stained long forms were practically absent and there were a large number of chainlike beaded forms and short cocco-bacillary forms began to appear. In the Koch-Novy strain the cocco-bacillary forms predominated at the one-month interval, the changes in this organism having run a more rapid course than with the other forms of rapidly growing acid-fast microorganisms in which the coccus and short bacillary forms began to predominate only



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Photomicrograph Series C.

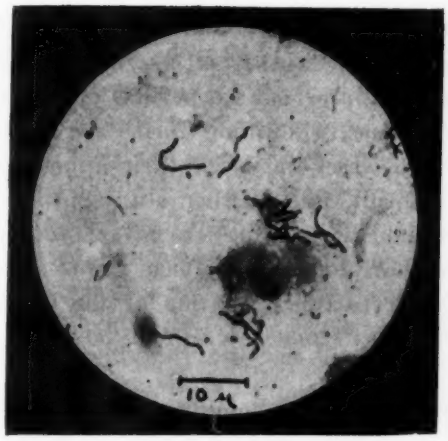
Cytomorphosis of Smegma bacilli grown on glycerol agar at 37° C.
1. Appearance of bacilli after incubation for one day.
2. Appearance of bacilli after incubation for one week.
3. Appearance of bacilli after incubation for one month.
4. Appearance of bacilli after incubation for four months.

after two and four months' cultivation at 37° C. During the course of this study, cultures were also examined which had been incubated at 37° C. for one week and then had been kept at room temperature in a dark box for two years. Many of these, although showing macroscopic culture, revealed upon

microscopic examination only a few coccus forms and large numbers of acid-fast globules. These various changes are well depicted in the appended illustrations.

In the study of the pathogenic varieties of tubercle bacilli, there were included two human and two bovine strains, all slow-growing cultures but differing in virulence for the guinea pig. One strain "Human" was a relatively avirulent strain of human tubercle bacilli producing a generalized tuberculosis in the guinea pig by injection only in amounts exceeding 1 to 10 milligrams and growing rather sparsely at 37° C. on glycerol-agar and egg mediums within two months. The other human strain "Gluckson" was a highly virulent organism producing a generalized disease in the guinea pig when injected in amounts as low as 0.000,000,001 milligram and growing profusely at 37° C. on glycerol-agar and egg mediums in two months. Both bovine strains ("Bov. D." and "Bov. Vir.") grew profusely on glycerol-agar and egg mediums within two months, and one ("Bov. Vir.") was highly virulent to guinea pigs, infecting in amounts less than 0.000,001 milligram, while the other ("Bov. D.") was moderately virulent. Since there occurred barely an appreciable growth with these strains before three weeks at 37° C., the first smears were made from cultures on glycerol-agar and Petroff's gentian-violet egg medium at this interval and after six weeks, three months, six months, and nine or ten months. All culture tubes were seeded from fairly well-grown parent cultures about one to two months old. Suspensions were made in saline solution and smears prepared as described for the rapidly growing bacilli above.

As a whole, except for the slow and gradual course of the changes, the same morphologic transformations with age occurred in the cultures of the slow-growing tubercle bacilli as were seen with the rapidly growing acid-fast forms. The less profuse growing "Human" culture did not reveal the long bacillary forms as seen in the cultures of the more profuse growing human strain "Gluckson" or the bovine strains but otherwise there was found a fair uniformity in the changes as they occurred with prolonged cultivation up to ten months. The long filamentous uniformly staining bacilli (in length up to 10 microns) predominated in the early periods of cultivation up to six weeks to three months, after which the shorter forms (3 to 5 microns) appeared and finally, at nine to ten months, the short bacillary (1 to 3 microns) and coccoid acid-fast forms predominated. It is noteworthy that in old glycerol broth cultures of tubercle bacilli which had remained in the incubator at 37° C. for as long as four years, short bacillary and coccoid acid-fast forms still persisted. It is interesting also that, although fewer in number at any certain period of examination, the length (8 to 10 microns) of some of the bacilli from the virulent human strain "Gluckson" on glycerol-agar or egg medium equalled that seen for the rapidly growing forms on the same mediums, making it seem reasonable that the rate of growth was accountable for the greater preponderance of the long forms at any one time during the early periods of growth in the rapidly growing culture.



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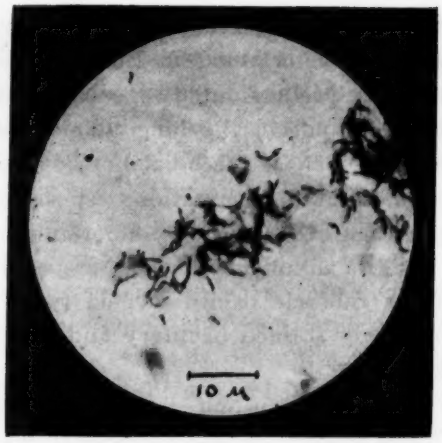

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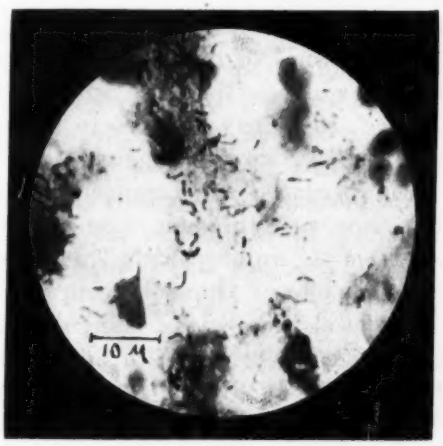

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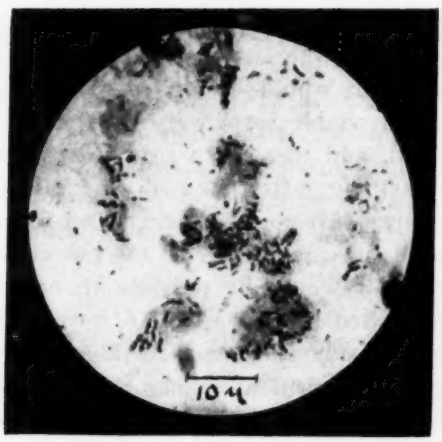

5.

Photomicrograph Series D.

Cytomorphosis of virulent human tubercle bacilli ("Gluckson") grown on glycerol agar or gentian-violet egg medium at 37° C.

1. Appearance of bacilli after incubation for three weeks.
2. Appearance of bacilli after incubation for six weeks.
3. Appearance of bacilli after incubation for three months.
4. Appearance of bacilli after incubation for six months.
5. Appearance of bacilli after incubation for ten months.

No consecutive sequence of the appearance of polar bodies could be definitely determined in the foregoing culture studies, although it seemed that they predominated especially during the intermediate periods of growth, occurring not in the long filamentous uniformly staining forms nor in the coccoid or short cocco-bacillary forms. The irregularity of their appearance made exact determinations especially difficult, and further study, probably by means of special specific staining methods, is required to attach to them a definite significance. Unlike spores in spore-forming bacteria, the tendency of the tubercle bacilli seemed to be rather to the formation of the shorter uniformly stained bacillary and coccoid forms from which polar bodies were lacking.

Having thus noted a definite sequence of morphologic growth changes in the rapidly growing acid-fast microorganisms studied which resemble in the main those occurring in the genuine human and bovine tubercle bacilli growing slowly on culture mediums, the second part of this study was concerned with the correlation of the culture studies to the changes occurring in human and bovine tubercle bacilli in the animal body. For this purpose the same two human and two bovine strains of tubercle bacilli were injected subcutaneously into guinea pigs. The experimental details will not be presented here except in brief, as they serve no particular end and add nothing to the clarity of the presentation of the problem. For the sake of uniformity, all the cultures used had been grown for six weeks at 37° C., the period when most active and profuse growth had attained. Stained smears were prepared from all cultures used for inoculation at the time of inoculation to serve as controls for the changes occurring in the body. Bacilli from both glycerol-agar or Petroff's gentian-violet egg medium were injected subcutaneously into normal guinea pigs in amounts ranging from 0.1 to 100 mg. of a uniform suspension in 0.5 to 2 c.c. of 0.9 per cent sodium chloride solution for the purpose of obtaining as wide a variation as possible in the concentration of bacilli in the tissues so that no morphologic variations would be missed in the study. In addition the same suspensions used in viable form were subjected to 100° C. for thirty minutes to kill the bacilli, and were then injected subcutaneously in amounts ranging from 50 to 200 mg. after stained smears had been prepared for comparison with smears of living suspensions and for comparison with the stained smears prepared subsequently from the pus obtained from the guinea pigs. Thus it was hoped it would be possible to note any morphologic changes occurring in heat-killed bacilli capable of producing only local tuberculous changes in the animal body and compare them with the changes occurring with viable avirulent and virulent human and bovine tubercle bacilli, the latter being capable of multiplication at different rates in the body and producing a generalized disease with fatal termination in the case of the virulent forms within a short period (one to two months) of time. In those cases in which pus developed and it was possible to obtain sufficient for the purpose, stained smears were prepared five, eight, eleven, twenty, thirty, fifty and ninety days after subcutaneous injection of the suspension of the bacilli. The staining technic

followed throughout this series was identical and uniform in order to rule out possible errors from this source. Likewise smears were made as nearly uniform as possible. The majority of the guinea pigs receiving the injections of the highly virulent strains of tubercle bacilli ("Gluckson" and "Bov. Vir.") died within thirty to fifty days so that observations beyond this were not possible, while those receiving the avirulent ("Human") and heat-killed bacilli lived well beyond the ninety-day period and were examined after this. Microscopic examination of the pus taken from the local site of injection into the guinea pig after fifty days reveals so few bacilli or is entirely negative, so that interpretation becomes difficult if not valueless,[5] and in the case where highly virulent bacilli were used the animals did not survive far beyond the thirty-day interval. Thus this experiment might be considered to have failed in attaining its full purpose, yet the results noted may be considered of some significance and will be given only in brief here. In all the guinea pigs that lived and from which pus was obtainable, there usually were numerous bacilli present in the stained smears up to the twentieth day. Although there was a marked variation in size of the individual bacilli seen, the range in variations and forms, regardless of whether dead bacilli or living bacilli had been injected, corresponded closely to that of the six weeks' old culture injected originally, there being a few long forms, but the predominating form was the intermediate shorter bacillary forms common to human tuberculous sputums. Many possessed metachromatic granules and some had polar bodies, but acid-fast coccoid or very short bacillary forms were not seen. Likewise the long strings of individual metachromatic granules were absent, the granules being noted only in the fainter stained bacilli. Regardless of whether the bacilli were injected in viable or heat-killed form, twenty to thirty days after injection into the guinea pig the pus contained so few bacilli that they were only found with difficulty, if at all. An examination of a large number of specimens from many animals only leaves one with the impression that the forms seen are those, not of marked accelerating growth or of senescence or the beginning of the death period, but the adult form of the period of negative acceleration according to Henrici. Further studies on the tubercle bacillus and the allied acid-fast microorganisms in its class will be required to elucidate more fully the forms predominantly found in tuberculosis as it occurs in man and animals, as well as to determine the significance of the metachromatic granules and the polar bodies found in certain forms of the bacilli.

DISCUSSION

The tubercle bacillus, in common with a number of nonpathogenic microorganisms of like type, possesses the property of acid-fastness, and, except for differences in rate of growth and animal pathogenicity, there is very little to serve as differentiating features. Three varieties of the acid-fast microorganisms (the human, bovine, and avian tubercle bacilli) possess pathogenicity and morphologically they resemble each other closely, so closely that it is doubtful whether one can differentiate them with any degree of accuracy by microscopic morphologic examination. Biologically the different

species differ markedly, and it is by biologic methods that our present classifications and differentiations have been possible. Animal pathogenicity, optimum growth temperatures, rate of growth and reaction curves have served in this. There have been many attempts to alter the tubercle bacillus culturally and morphologically by utilizing various modified mediums, chemical agents and unnatural conditions, but this has led only to an apparent temporary transformation, with immediate reversion upon restoration to one of the standard mediums and conditions of growth. These temporary morphologic transformations (involution forms) become perfectly clear when one recognizes that the closely allied acid-fast organisms undergo certain progressive changes in growth which can be varied, as pointed out by Henrici, by any factors interfering with the normal growth curve or by the time at which the examination occurs. The acid-fast microorganisms included in this study, three rapidly growing acid-fast strains, an avian strain, two human strains and two bovine strains, revealed approximately the same morphologic changes—cytomorphosis—even though the rate of growth was markedly different for the individual strains. In the culture tube under suitable environment (temperature, culture medium, etc.) the bacillus can undergo morphologic transformation which when duly considered from the time standpoint give us the consecutive stages in its development. The acid-fast microorganisms, studied culturally in the light of the recent contributions by Henrici, make it evident that the young forms consist of uniformly-staining, long, straight and curved acid-fast filaments, the adult forms being shorter bacillary forms with metachromatic granules and the senescent form being uniformly stained acid-fast coccoid and very short bacillary forms. This is in entire accord with the conceptions of Wolbach and Ernst, who studied only human and bovine cultures of tubercle bacilli. A correlation of this with the animal studies makes it appear evident that the usual form seen in pus from experimental tuberculosis in animals (guinea pigs) is the adult form of tubercle bacilli; only occasionally is the young or embryonic form seen, and extremely rarely if at all the senescent form. It appears also that the adult form is the usual form encountered in tuberculous sputums from open cases of pulmonary tuberculosis.*

SUMMARY

Rapidly developing acid-fast nonpathogenic tubercle bacilli (Smegma, "Koch-Novy," and "Day") and rapidly and slowly developing pathogenic tubercle bacilli (human, bovine and avian), grown upon glycerol-agar and egg mediums, undergo a regular metamorphosis—cytomorphosis (Henrici)—which can be correlated with the time and growth curve of the bacilli. The period of early (accelerating) growth is identified with the presence of a large number of long filamentous, uniformly staining, acid-fast bacilli, while the period of adult (phase of negative acceleration) growth is identified with a preponderance of moderately long bacillary forms with and without metachromatic granules, and the senescent cells consist of uniformly acid-fast

*Note. For kind assistance in performing the technical details of this study the author is grateful to P. Cosman and Mary Moore.

coccoid and short bacillary forms. Correlated with macroscopic observations and cultural transplant studies, for slow growing human and bovine tubercle bacilli, the young embryonic forms were evident during the active period (first few months) of macroscopic growth on glycerol-agar and egg mediums,[12] while the senescent forms predominated (after eight to nine months), when subculturing to these mediums became more difficult or unsuccessful.

The tuberculous pus resulting from the subcutaneous injection of large amounts of living or heat-killed human or bovine tubercle bacilli (from a culture six weeks old) into guinea pigs, revealed predominantly the adult forms of tubercle bacilli. There was a gradual decrease in the number of bacilli present microscopically in the pus whether produced by living or heat-killed bacilli until that obtained after fifty days contained only a few bacilli or was negative. During the period of observation possible, there did not seem to be any change in relations of the forms of bacilli present in the pus at the local site of injection, and the virulence of the strain seemed to play no part, although the animals infected with the virulent strains died of a generalized tuberculosis within approximately one month after infection. Further and more exhaustive experiments are, however, necessary to be conclusive on this point and to elucidate this problem in so far as it concerns tuberculosis in man. It appears that the adult form of human tubercle bacilli[18] predominates in the sputum, as seen in routine examinations, from the majority of cases of pulmonary tuberculosis in man.

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EPINEPHRIN SHOWN CHEMICALLY IN THE BLOOD. DETERMINATION DIFFERENTIATING EPINEPHRIN FROM GLUCOSE IN THE BLOOD

(Preliminary Report)

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THE blood-sugar field is at present very well cultivated. Still, two very striking points present themselves: the clinician is not satisfied with the present interpretation of blood-sugar findings; and the literature testifies to the uncertainty of the whole subject. The object of this paper is to show by chemical means the presence of epinephrin in both normal and pathologic blood-sugar findings. An attempt will be made to demonstrate that in many cases of apparent "hyperglycemia" we are actually estimating epinephrin and not sugar.

ABSTRACTS FROM LITERATURE

Lundsgaard and Holboell,[1] in an investigation of eleven normal subjects showed that a part of the blood sugar exists in a form possessing a specific rotation weaker than in A and B glucose. This was manifest during both fasting and digestion. This form of glucose is similar to the substance obtained in vitro from A and B glucose under the effect of insulin or fresh muscle tissue.

In a second report[2] these investigators present evidence that the change in the specific rotation of light by a solution of A and B glucose after the addition of fresh muscle tissue and insulin is due to the production of a new form of glucose with a low specific rotating power. They explain it as the first phase of carbohydrate metabolism and call it the "new" glucose.

Winter and Smith[3] have recently advanced the suggestion that the sugar in normal blood is the highly reactive isomer, A-glucose, while in cases of severe diabetes the blood sugar is the relatively stable B form. They based this hypothesis upon observations that deproteinized extracts of normal blood when examined polariscopically over a period of three days show a progressively decreasing rotation. The power of these same extracts to decolorize potassium permanganate also decreases. Deproteinized bloods of severe diabetics, on the other hand, showed this phenomenon not at all or to a greatly lessened degree.

In another paper, Winter and Smith[4] present a note upon work done upon rabbits made hyperglycemic by means of epinephrin injections. In this paper they state that in this condition the mixture of sugars present in the blood would appear to be similar to that existing in diabetic blood before the injection of insulin. They advance the theory that the cause of diabetes is the absence or inactivation of an enzyme whose function it is to transform the relatively stable A B glucose into the exceedingly unstable A form.

Visscher[5] corroborated the findings of Winter and Smith with normal blood but found that the difference between normal and diabetic blood could be determined by varying the hydrogen-ion concentration of the deproteinized extract.

Denis and Hume,[6] upon repetition of the work, consider the claims of Winter and Smith unjustifiable and lacking in proof.

Hiller, Linder, and Van Slyke,[7] after subjecting blood to yeast fermentation and incubation at 38° C. under conditions that lead to complete destruction of glucose, conclude that there is a residue of a reducing substance in the blood other than glucose.

Physiologically, Tournade and Chabrel,[8] after having joined the suprarenal vein of one dog to the jugular vein of another, noted that centripetal irritation of the sciatic or crural nerve in the donor induced in the other dog a slight hyperepinephrinemia. The latter was manifested by contraction of the spleen, with or without changes in the arterial pressure.

In a later paper[9] they describe experiments upon dogs with one or both suprarenal glands removed and affirm that the amount of epinephrin poured out into the blood by a normal gland plays an unmistakable physiologic rôle in the organism.

Gley and Quinquad's[10] research upon cats showed that venous blood drawn from the suprarenal glands during irritation of the splanchnic nerve, manifested the properties of epinephrin when reinjected into the same animal.

The determination here described is based upon the fact that epinephrin reacts with picric acid and sodium carbonate with heat, producing a red color identical with that given by glucose. Since both glucose and adrenalin give this same red color, it is obvious that one must be removed. Iron is absolutely destructive to epinephrin and is, therefore, employed to meet this end. Two sugar determinations are run one with, the other without iron, the former being the true sugar content.

EXPERIMENTAL

Purpose.—To show that ferric chloride destroys epinephrin but has no effect upon pure glucose.

Procedure.—A solution of glucose was made at random, about 0.3 per cent strength from the c. p. crystals. A solution of adrenalin in 0.5 per cent hydrochloric acid was made from the c. p. crystals (Parke, Davis and Company). A few crystals from a one-grain vial were dropped into a test tube, quantity indefinite.

(Iron wherever used was filtered out in the following manner: A small 2.5 cm. funnel was employed. Flask contents were emptied into the filter, which were permitted to drain completely into a volumetric flask. They were washed with 1 or 2 c.c. of water which was added to the original flask, rotated well, and then poured into the funnel, washing the paper carefully. The funnel was allowed to drain completely before further washings were added from the original flask. About 4 or 5 washings were required, the funnel being emptied completely each time before the addition of further washings. The solution was cooled and then made up to volume. The last filtrates came through water-clear, and the paper was absolutely free from any color of the solution.)

1a. Exactly 1 c.c. of the glucose solution was pipetted into a 150 c.c. Erlenmeyer flask. Exactly 10 c.c. of saturated aqueous solution of picric acid were added, followed by 2 c.c. of 10 per cent sodium carbonate. Contents were boiled over a small, free flame almost to dryness; they were then transferred quantitatively to a 25 c.c. volumetric flask, cooled, and made up to mark.

1b. Same as above, except that a few small pieces of ferric chloride were added before the addition of the sodium carbonate. The final solution was heated and filtered.

2a. Exactly 3 c.c. of the adrenalin solution were pipetted into a similar flask and treated as in *1a*.

2b. Same as *2a*, except that ferric chloride was added before the addition of the sodium carbonate. The final solution was heated and then filtered.

3a. Exactly 1 c.c. of the sugar solution and 3 c.c. of the adrenalin solution were treated as in *1a*.

3b. Same as *3a*, except that ferric chloride was added. The final solution was then heated and filtered.

The solutions were compared with each other.

STANDARD		READINGS	
1a. set at	10.00 mm.	*1b*	10.00 mm.
2a. " "	10.00 mm.	*2b*	11.00 mm.
1a. " "	10.00 mm.	*3a*	9.00 mm.
1a. " "	10.00 mm.	*3b*	10.00 mm.

Fearing that some of the adrenalin might have been destroyed in the acid solution, the above was repeated as follows:

The same glucose solution was used. Adrenalin crystals were dropped into a beaker containing 10 c.c. of saturated picric acid solution. Solution is instantaneous. Five c.c. of this solution were transferred to the Erlenmeyer flask, and 5 c.c. additional picric acid were added, making 10 c.c. in all. The remaining 5 c.c. were used in the twin test. Procedure the same as the previous. Comparisons:

STANDARD		READINGS	
1a. set at	10.00 mm.	*1b*	10.00 mm.
2a. " "	10.00 mm.	*2b*	17.90 mm.
1a. " "	10.00 mm.	*3a*	5.50 mm.
1a. " "	10.00 mm.	*3b*	9.70 mm.
	3a and *3b* were compared		
3a. set at	10.00 mm.	*3b*	14.20 mm.

This shows conclusively that sugar is not affected.

WORK DONE ON HUMAN BLOOD

After having determined the glucose and the epinephrin content of various human bloods, glucose or adrenalin (Parke, Davis and Company), and in some cases both, were added and studied as indicated. In all this work no attention is paid to the quantity of either substance added, for the reason that glucose is known to react quantitatively, the object being to show: first, that added adrenalin intensifies the color of the sugar reaction; and, second, that adrenalin is destroyed by ferric chloride. See Table I.

TABLE I

NO.	TREATMENT OF BLOOD	READING	DILUTION	PRESENT FIGURE AS IS EPINEPHRIN AND GLUCOSE	READING	DILUTION	EPINEPHRIN DESTROYED—GLUCOSE ONLY
		mm.	c.c.	mg.	mm.	c.c.	mg.
1	Ppt. 15 minutes after bleeding	3.9	10	256.4	4.0	10	250.0
	Adrenalin added, part ppt., immediately	7.2	25	347.0	3.4	10	294.1
	Blood containing adrenalin left standing at room temperature for 24 hours	3.7	10	270.2			
2	Ppt. 15 minutes after bleeding	3.8	10	263.1	3.9	10	256.4
	Left standing as is room temperature for 24 hours	15.5	10	64.5			
	After standing adrenalin added, ppt., immediately	4.8	10	208.3	7.0	10	142.8
3	Ppt. 30 minutes after bleeding	7.1	10	140.8		Lost.	
	Glucose added to one portion, ppt., immediately	3.6	25	694.2	3.6	25	694.2
	Adrenalin added to other portion, ppt., immediately	1.4	25	1785.5	5.1	10	196.0
4	Ppt. one minute after bleeding	6.8	10	147.0	7.4	10	135.1
	Glucose and adrenalin added, part ppt., immediately	2.8	25	892.7	3.2	25	781.2
	Blood with added sugar and adrenalin left standing room temperature 24 hours	1.7	10	588.2			
5	Ppt. 5 minutes after bleeding	13.2	10	75.7	15.6	10	64.1
	Glucose and adrenalin added, ppt. at once	3.2	25	781.2	4.6	25	543.2
	Same left standing room temp., 24 hr.	4.1	25	609.7	4.1	25	609.7
	Same left standing room temp., 48 hr.	14.1	10	70.9	14.1	10	70.9
6	Ppt. 15 minutes after bleeding	7.3	10	136.9		Lost.	
	Glucose and adrenalin added, part ppt., immediately	4.1	10	243.9	5.5	10	181.8
	Left standing room temp., 24 hr.	4.3	10	232.5	4.5	10	222.2
	Same left standing room temp., 48 hr.	18.7	10	53.4			
7	Ppt. 30 minutes after bleeding (diabetes)	3.8	10	263.1	3.8	10	263.1
	Left standing room temp., one week; glucose and adrenalin added, ppt., immediately	1.5	10	666.6	2.3	10	434.7
	Same left standing room temp., 24 hr.	3.1	10	322.5	3.1	10	322.5
	Given insulin, bled one month later, as is	3.3	10	303.0	3.3	10	303.0
	Left standing room temp., 24 hr.	3.5	10	285.7			
	After standing, adrenalin and glucose added, ppt., immediately	3.3	50	1515.0	2.4	10	416.6
	Left standing room temp., 24 hr.	3.5	10	285.7	3.5	10	285.7
	Left standing room temp., 48 hr.	6.4	10	156.2			
	Left standing room temp., 60 hr.	6.4	10	156.2			
	Bled one month later, on insulin	4.7	10	212.7	5.3	10	188.6
8	Ppt. one minute after bleeding	11.5	10	86.9	20.0	10	50.0
	Glucose added, ppt., immediately	5.1	10	196.0	5.1	10	196.0
	Left standing room temp., 48 hr.	7.0	10	142.8			
	Adrenalin added, ppt., immediately	6.1	25	409.7	10.0	10	100.0
	Left standing room temp., 48 hr.	Color nil.					
9	Ppt. 10 minutes after bleeding	2.6	10	384.6	2.8	10	357.1
	Left standing as is room temp., 24 hr.	2.6	10	384.6			
	Left standing 48 hr., room temp.	2.6	10	384.6			
10	Plasma separated and left standing at room temperature one week						
	To part, glucose was added, ppt., immediately	1.3	50	3846.0	1.3	50	3846.0
	Left standing room temp., 24 hr.	1.6	50	3125.0			
	To other part adrenalin was added, ppt., immediately	5.0	10	200.0	8.2	10	121.9
11	Plasma separated, glucose and adrenalin added, ppt., immediately	2.7	10	370.3	4.1	10	243.9
	Left standing room temp., 24 hr.	2.7	10	370.3	4.1	10	243.9
	Left standing 48 hr.	5.8	10	172.4	8.4	10	119.0

TABLE II

	SUBJECT	BLOOD PRESSURE	BLOOD SUGARS			
			READING FIRST TEST	PRESENT FIGURE AS IS EPINEPHRIN AND GLUCOSE	READING SECOND TEST	EPINEPHRIN DESTROYED GLUCOSE ONLY
1	*Female adult*	mm.	mm.	mg.	mm.	mg.
	Patient had had supper at 7:00 P.M.					
	First blood drawn at 8:30 P.M.	117.7	11.3	88.4	15.6	64.1
	10 minims injected: 5 minutes, B. P., 135.0 mm.; began to lower					
	10 minims more injected: 2 minutes	132.0				
	Second blood		9.5	105.2	12.7	78.7
2	*Male adult* (Addison's disease)					
	Patient had had supper at 7:00 P.M.					
	First blood drawn at 10:00 P.M.	105.0	11.4	87.7	16.3	61.3
	30 minims injected: 3 minutes	160.0				
	Second blood		7.2	138.8	14.0	71.4
3	*Male adult*					
	Fasting stomach 9:00 A.M., 1" blood	105.0	9.1	109.7	9.1	109.7
	10 minims injected; 5 minutes	150.0				
	Second blood		6.6	151.5	8.4	119.0
4	*Male adult*					
	Fasting stomach 9:00 A.M., 1" blood	90.0	10.4	96.1	15.0	66.6
	20 minims injected: 5 minutes	164.0				
	Second blood		6.1	163.9	13.1	76.3
5	*Female adult*					
	Patient had had supper at 6:00 P.M.					
	First blood drawn at 8:30 P.M.	85.0	11.3	88.4	16.1	62.1
	20 minims injected; rise to 120.0 mm., immediate drop. 5 minutes, 20 minims more injected: 3 minutes	136.1				
	Second blood		8.0	125.0	13.9	71.9

A few patients received injections of adrenalin during their regular course of treatment. In this work adrenalin (1:1000 P. D.) was used. Injection was made into the buttocks. Blood was drawn from the median basilic vein. A Tycos sphygmomanometer was kept on the arm throughout the entire procedure. All of the blood sugars were precipitated immediately, necessary apparatus and reagents having been taken to the bedside when the patient was not ambulant.

From Table II it appears that hyperepinephrinemia and not hyperglycemia results from the injection of epinephrin. Increase of color density and rise of blood pressure are shown. The recovery of epinephrin from the blood of the patient is also demonstrated. In this connection, a recent work of Ohara[11] is of particular interest. In rabbits whose liver glycogen was made to disappear almost entirely through starvation, injections of epinephrin were noted to cause a very conspicuous *hyperglycemia.* Repeated injections of adrenalin developed a *glycosuria,* and marked *hyperglycemia.* Ohara also produced a marked *hyperglycemia* in hepatectomized rabbits. Inference is made by Ohara, that the

hyperglycemia produced in these hepatectomized animals might be due to the breakdown of the glycogen in the muscles. Ohara's work appears to confirm the results just given that hyperepinephrinemia and epinephrinuria are produced by injections of adrenalin.

METHOD

It is important that the blood be precipitated immediately, or as soon as possible, after bleeding, if the results are to be of any value.

Pipette 2 c.c. whole oxalated blood into a 25 c.c. volumetric flask. Add a saturated aqueous solution of picric acid and make up to mark. Shake well to insure thorough mixing, filter. Two 8 c.c. aliquots are required. Measure one 8 c.c. portion into a 150 c.c. pyrex Erlenmeyer flask. Add 2 c.c. of 10 per cent sodium carbonate solution and boil over a small, free flame until precipitation occurs. Dissolve in 1 or 2 c.c. of water, transfer quantitatively to a 10 c.c. volumetric flask, cool, and make up to volume. Pipette the other 8 c.c. aliquot into a similar flask, add about 50 or 80 mg. Fe_2Cl_6 (about 2 or 3 small pieces). Add 2 c.c. 10 per cent sodium carbonate solution and boil as before.

Add 1 or 2 c.c. water, filter through a small 2.5 cm. funnel into a 10 c.c. volumetric flask. Permit the funnel to empty completely; add 1 or 2 c.c. water to the original flask, and pour into the funnel, carefully washing the paper. Repeat until almost up to mark, allowing complete filtration each time before adding washings. Cool and make up to mark. This takes about 4 washings. When the operation is complete, the paper should be absolutely free of any color from the solution, and the filtrate should come through water-clear.

Comparisons should be made at once in any standard colorimeter. Standard blood-sugar solution* is used and is set at 10.00 mm.

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 100 = \text{mg. per 100 c.c.}$$

Reading of first determination is sugar and epinephrin. Reading of second determination is sugar only (epinephrin has been destroyed).

Color equivalent for epinephrin has not as yet been determined. This test is simply to differentiate between the actual sugar content and that color which is due to epinephrin. Attention is here called to an observation throughout this work of a slight increase over the actual sugar figure after the added or injected epinephrin has been destroyed. No explanation is offered for this as yet. Following is a list of some of the unselected cases studied. All diagnoses were made by the clinician.

DISCUSSION

While glucose reacts with certain reagents which form the basis of blood analytic procedures, it is recognized that none of them is entirely specific for glucose. In various conditions, as for example, severe nephritis, hypertension (some forms), certain cardiac conditions and hyperthyroidism, there is an

*Permanent Standard

Picramic acid	0.064 gm.
Na_2CO_3 (anhydrous)	0.100 gm.
Distilled water to mark	1000.000 c.c.

Standardize against pure glucose.

TABLE III

CASE NO.	SEX	CONDITION	READING FIRST TEST	PRESENT FIGURE AS IS, EPINEPHRIN AND GLUCOSE	READING SECOND TEST	EPINEPHRIN DESTROYED—GLUCOSE ONLY
1492	M	Diabetes mellitus	3.9	256.4	4.0	250.0
1523	F	Diabetes mellitus	3.8	263.1	3.9	256.4
1249	M	Diabetes mellitus (insulin case)	3.7	270.0	5.3	188.6
1306	M	Diabetes mellitus (treated) nephritis	2.4	416.4	2.6	384.6
1384	F	Diabetes mellitus (treated) pyelitis	2.7	370.0	3.3	303.0
1786	F	Diabetes mellitus, essential hypertension	3.4	294.1	6.2	161.2
1326	F	Diabetes mellitus, 3/20/24	4.9	204.0	Lost	
		Same, 3/31/24	3.8	263.1	3.8	263.1
		Same, 4/16/24	2.4	416.6	2.4	416.6
		Same, 4/30/24, insulin started	3.3	303.0		
		Same, 5/13/24, gangrene, exitus	4.7	212.7	5.3	188.6
2074	M	Diabetes mellitus, after treatment	3.3	303.0	5.0	200.0
2742	F	Diabetes mellitus, B. P., 200 mm.	3.5	285.7	7.4	135.1
	M	Diabetes mellitus, neuritis (reading x 2)	6.5	309.2	6.5	309.2
3447	F	Diabetes mellitus (changed doctors)	2.6	384.6	2.8	357.1
3372	F	Diabetes mellitus (insulin)	4.2	238.0	7.8	128.2
1467	F	Toxic hyprtension, B. P. 200/130, obesity, nephritis, hyperthyroidism	3.0	333.3	3.5	285.7
1410	F	Essential hypertension, B. P., 195/100, arthritis	4.5	222.2	9.6	104.1
1336	M	Essential hypertension, mild pyelitis	7.1	140.8	9.3	107.5
1558	M	Hypertension, occasional glycosuria	7.1	140.8	Lost	
1431	F	Questionable diabetes, hypertension	6.5	153.8	7.0	142.8
2029	F	Nephritis, arthritis, essential hypertension	5.8	172.4	11.7	85.4
3228	M	Essential hypertension	5.2	192.3	16.0	62.5
1494	F	Polyarthritis, possible hyperthyroid	2.9	344.8	2.9	344.8
1318	F	Arthritis, nephritis, hyperthyroidism	4.8	208.3	4.8	208.3
1631	M	Obesity, alcoholism	5.4	185.1	6.0	166.6
3517	F	Arthritis, hypertension	2.5	400.0	4.6	217.3
1645	F	Arthritis, autointoxication	9.3	107.5	11.8	84.7
1614	F	Hernia	7.7	129.8	7.7	129.8
1764	M	Fatigue, no diagnosis to date	6.2	161.2	10.2	98.0
1618	F	No diagnosis	6.8	147.0	7.4	135.1
1672	M	No diagnosis	9.8	102.0	11.7	85.4
1754	M	Nephritis, hypotension	13.2	75.7	15.6	64.1
1864	F	No diagnosis	10.4	96.1	13.7	72.9
1872	M	No diagnosis	10.9	91.7	14.5	68.9
1778	F	Arthritis, hypotension	12.1	82.6	17.5	57.1
1869	F	Chronic supp. nephritis, arthritis, infantile uterus	10.8	92.5	15.4	64.5
1882	M	Asthenia, vertigo, hypotension	10.9	91.7	10.9	91.7
1897	M	No diagnosis	7.8	128.2	14.7	68.0
1909	F	No diagnosis (hypotension)	12.1	82.6	15.2	65.2
1947	M	Arthritis, toxemia, mild nephritis, hypotension	11.4	87.7	15.0	66.6
1964	M	Addison's disease, possible Ca . . ?	10.7	93.4	11.5	86.9
		Same two month's later.	11.4	87.7	16.3	61.3
		Same 2 weeks later, died—Addison's	21.9	45.6	21.9	45.6
1988	F	Toxic hypertension, pyelitis, (tonsils, teeth)	10.2	98.0	14.5	68.4
1990	M	Lymphatic leucemia	9.6	104.1	15.5	64.5
2038	F	Essential hypertension	7.2	138.8	13.8	72.4
1818	F	Skin lesion, mild hypertension	7.4	135.1	11.3	88.4
1546	M	Asthenia, hypotension, possible Ca . . ?	10.8	92.5	10.8	92.5
2754	F	No diagnosis	9.2	108.6	16.4	60.9
2856	F	No diagnosis	9.9	101.0	21.6	46.2
3189	F	Fatigue, hypotension	11.3	88.4	20.1	49.7
3325	F	Arthritis	9.6	104.1	28.0	35.7

TABLE III—CONT'D

CASE NO.	SEX	CONDITION	READING FIRST TEST	PRESENT FIGURE AS IS, EPINEPHRIN AND GLUCOSE	READING SECOND TEST	EPINEPHRIN DESTROYED—GLUCOSE ONLY
3318	M	Moderately increased blood pressure	8.3	120.4	14.2	70.4
	F	Hypertension	7.3	136.9	13.5	74.0
3399	M	No diagnosis	8.5	117.6	10.5	95.2
3469	M	No diagnosis	9.2	108.6	20.5	48.7
3505	M	Glycosuria	9.5	105.2	15.0	66.6
3478	M	Asthenia, chronic bronchitis, tonsils, hypotension, B. P. 90/50	9.3	107.5	13.8	72.4
3527	F	Arthritis?	2.5	400.0	4.6	217.3
3527	M	Asthenia, hypotension, toxemia, tonsils (following tonsillectomy, B. P. normal)	9.7	103.0	13.8	72.4
3541	M	Gastric ulcer	10.1	99.0	15.0	66.6
1662	M	B. P. 200/150	11.4	87.7	12.2	81.1
3661	F	Very obese, tonsils, possible growth?	9.8	102.0	15 0	66.6

hyperglycemia which has no explanation. Epinephrin, as shown in another paper,[12] reacts with picric acid and gives all the reduction tests identical with those of glucose. Since, therefore, as shown in this paper, it increases the color density of the blood sugar, and since it is known to cause a rise in blood pressure, it seems conclusive, in some instances at least, that we are estimating epinephrin and not glucose.

A discussion on diabetes is entirely too vast and out of the scope of this paper; still a few remarks will not be amiss. The pancreatic theory of diabetes has by far the greater number of followers and, with the discovery of insulin, the long surmised pancreatic internal secretion controlling carbohydrate metabolism has been confirmed; still the pancreas as the seat of structural alteration is questionable, for, according to Allen,[13] it is striking how much degeneration of the pancreas can be produced without diabetes, in contrast to the relatively slight changes found in diabetic men and animals. Epstein[14] states that while it is believed that the disease is due to a deficient production of insulin, the exact mechanism of the production of this condition is still problematic. This disease might, then, be the effect rather than the cause.

Returning to the subject of this communication, a prominent point relative to diabetes suggests itself. I do not want to be too emphatic in stressing this point, but from mere observation, it would appear that the epinephrin content of the blood stream in pancreatic diabetes is nil and on insulin treatment, with the decrease in the glucose content, there is a return of epinephrin to the normal and even to excessive quantity. Even in cases in which the blood sugar under insulin treatment cannot be decreased below a certain point, say 150-180 mg., the epinephrin content as per this test seems to rise, whereas the actual glucose content is greatly reduced, being normal in some cases and in others only slightly increased. No attempt is made to explain these phenomena. Can-

non, McIver and Bliss[15] and recently Müller and Petersen[16] have reported that the injection of epinephrin before insulin retards the marked decrease in the blood-sugar content. A coordination between the suprarenal glands and the pancreas might be suspected. I wish to emphasize again that the above is simply an observation based upon some 300 cases, which is entirely too small a number for conclusive deductions. It is simply included as a possible clue to the solution of the problem.

A change of the present normal blood-sugar figure is obvious. Benedict,[17] in discussing a new method, is of the opinion that the sugar content of blood averages about 75.0 mg. per 100 c.c., and that the actual glucose content of normal blood does not usually exceed 60.0 mg., except for a short period after the ingestion of food. From the data presented here, it would appear that the normal glucose content averages about from 55.0 or 60.0 mg. to about 75.0 or 80.0 mg. per 100 c.c., and that between 80.0 and 110.0 or about 20 mg. (terms of glucose) above the glucose content would be the normal epinephrin content.

Epinephrin is shown, contrary to accepted theory, to produce an hyperepinephrinemia and not hyperglycemia. This would then appear to confirm the supposition entertained by some that the cause of some forms of hypertension might be overactivity of the suprarenal glands.

SUMMARY

Epinephrin is shown to be a normal constituent of the blood stream. A determination is offered to differentiate between the epinephrin and the actual glucose content of the blood.

An attempt is made to demonstrate the difference between true hyperglycemia and hyperepinephrinemia; e. g., adrenalin added to human blood is shown to increase the color intensity of the blood-sugar reagent, which color is diminished almost to the color of the original sugar content after treatment with ferric chloride. (Pure glucose is shown to be unaffected by ferric chloride.) This is further shown by injections of adrenalin into human subjects. The blood pressure is shown to rise. There is an increase in the color density of the blood sugar and then upon addition of the Fe_2Cl_6 to the same filtrate the color density diminishes. Glucose does not react in this manner.

A new normal blood-sugar figure is suggested.

Such substances as uric acid, creatinine, creatine, glucuronic acid, lactose and purins, which might interefere (add to) with the blood-sugar determination were found to be unaffected after treatment with ferric chloride.

Attention is again called to the fact that the color equivalent of epinephrin has not as yet been worked out. Clinically, this will suffice for the present, for, if the *actual* glucose figure is determined, the excess color due to epinephrin, be it large or small, along with the clinical findings, is sufficient for diagnostic purposes.

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BASAL METABOLIC RATE IN SIMPLE AND PATHOLOGIC OBESITY*

By Horry M. Jones, M.D., Chicago, Ill.

WHY will two individuals, each in the same apparent state of good health, show widely different tendencies to gain in weight?

The so-called normal individual seems immune to all sorts of dietary indiscretions. In spite of his habits of spending his energies as he likes and eating as much or as little as he pleases, he shows no more than a pound or two of change in weight over long periods of years.

Another individual, otherwise equally normal, must urge himself to vigorous exercise, and must continuously inflict upon himself dietary sacrifices, and in spite of these restrictions his weight mounts up and up as the years go by.

This is the problem of simple obesity. In discussing the problem of pathologic obesity, it will be necessary to digress from the main topic long enough to try to agree on what kind of cases shall be classed as pathologic.

Law of Conservation of Energy. Energy may be transformed from one form to another but may not be created or destroyed.

There are four forms of energy: (1) electrical, (2) mechanical, (3) radiant, and (4) chemical. When a given quantity of any one of these four forms of energy is transformed into another form, an equivalent quantity of energy reappears, because, although energy is transformable, it may not be created or destroyed. This principle of conservation of energy is fundamental to a consideration of the subject of obesity.

Animal tissues are capable of transforming only one form of energy, namely, chemical energy. This chemical energy arriving within the tissues as food is transformable into three forms of energy: namely, (1) radiant, (2) mechanical, and (3) chemical.

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The animal tissues are, like the food itself, stored *chemical* energy. If the chemical energy of the food circulating through the tissues be *in excess* of the body's immediate needs for radiant and mechanical energy, this excess must be "resynthesized" into chemical energy, and stored up along with the other tissues, because their excess cannot be destroyed and cannot escape from the tissues.

If the energy of the food supplied to the tissues be *insufficient* to meet the body's constant needs for radiant and mechanical energy, then, since this needed energy cannot be created, it must be supplied by transforming some of the chemical energy previously laid down in the body in the form of body tissues.

In other words, the entire mass of the body's tissues is the result of chemical energy in the form of food reaching the tissues *in excess* of the body's constant needs for radiant and mechanical energy.

Likewise, when chemical energy is supplied to the tissues in amounts *insufficient* to meet the body's needs for mechanical and radiant energy, there must result a transformation of the stored energy of the tissues to make up this shortage and, therefore, a loss in weight corresponding to the weight of the tissues so consumed for that purpose.

The body tissues and the foods are latent chemical energy, but they are rated as radiant energy, i.e., in terms of calories. For example, the latent chemical energy in fats, proteins, and carbohydrates of our food intake is transformable, respectively, into 9.3, 4.1, and 4.1 calories of radiant energy per gram when these are oxidized in the tissues, and these figures represent the so-called food values of fats, proteins, and carbohydrates.

A pound of dried muscle protein, therefore, contains 454 grams × 4.1 calories or 1860 calories. But muscle protein resynthesized into muscle tissue contains only 30 per cent of this 1860 calories, i.e., only 560 calories per pound of body weight, for the reason that muscle is 70 per cent water. Fat tissue on the other hand contains 4200 calories per pound of body weight, i.e., 9.3 × 454 grams = 4200 calories.

In other words, the growth of a pound weight of muscle in the body requires an excess of only 560 calories in the food intake, whereas, if stored as a pound of fat, an excess of 4200 calories is required.

Conversely, to lose a pound of fat which has been stored in the body as excess energy requires a shortage in diet of 4200 calories.

A person having a 2400 outgo and a 2400 intake per twenty-four hours would, therefore, be obliged to endure a shortage in the intake of food of 600 calories per day for an entire week in order to lose a pound of weight of fat.

1.—This radical reduction of 600 calories in the diet will induce almost constant hunger. 2.—The malaise or loss of both mental and physical stamina induced by this so-called "dieting" invariably causes a slowing up of the above-mentioned 2400 calories outgo, and thus partially or completely offsets the desired 600 calorie shortage. 3.—Restricting the diet below the normally required 2400 caloric intake causes a more economical absorption from the

intestines of food particles (especially fats, some of which would normally have been wasted as feces), thus again tending partially or completely to offset the desired 600 calorie shortage. 4.—After having endured constant hunger for an entire week or more, the individual unacquainted with the above mathematics of dieting, expects the scales to show a loss in weight commensurate with his dietary sacrifices and the hunger he has suffered. 5.—If the individual is then weighed as before on the same scales with the same clothing, the pound or more loss in fat tissues even then may chance to be offset by the weight of material in an unevacuated bowel or bladder. 6.—And finally, on a weight reducing diet, the fat consumed is often replaced by an equivalent weight of water, which is retained in the tissues and may amount to as much as eight pounds before any evidence of it begins to appear as edema. In such an instance one could live on a 600 calorie daily shortage for two months, and still find on weighing, no measurable reduction in body weight.

To any one unfamiliar with the law of conservation of energy and the mathematics of metabolism, such painfully prolonged demonstrations would be sufficient proof that diet has nothing to do with the body weight. This opinion, namely, that controlling the diet does not control the body weight, is prevalent, everywhere. If it were not, there would be no profit in the various nostrums, systems, drugs, and devices offered to the public for reducing excess body weight.

Even in the medical press, obesity has as many theories to explain it as there are remedies to cure it. The popular theory is that obesity is an hereditary characteristic, as seen in certain breeds of farm animals. Development of the normal bony structure, the circumference and length of the muscles under normal exercise, and also the sites chosen by the body for the deposition of adipose tissues are undoubtedly hereditary characteristics, but the *amount* of fat storage is not determined by heredity.

Another theory is that the specific dynamic action of protein is decidedly less marked in the obese than in normal individuals so that the obese can subsist indefinitely "on intakes much below their calculated caloric requirements."

Metabolic rate determinations on fourteen cases of simple obesity, using six normal subjects as controls, all of which were given a meal containing known amounts of protein, fats, and carbohydrates, showed practically the same rise in the metabolic rate proportionate to the amount of food ingested.

For example, a woman of 290 pounds weight and another woman of 110 pounds weight, after taking a 1,000 calorie dinner, showed, respectively, losses of 13 per cent and 12 per cent of this 1,000 calories calculated from the time of ingestion of the meal until the metabolic rates had dropped back to the basal rate, which was about six hours. Therefore, 13 per cent and 12 per cent of 1,000 calories = 130 and 120 calories, respectively, to be distributed over six hours of time, the period of absorption, or 21 and 20 calories respectively, per hour.

But this 21 calorie per hour increase in the obese subject's metabolism was only a 22 per cent increase over her 94 calorie basal metabolic rate, whereas, the 20 calories per hour increase in the normal subject's metabolism was a 37 per cent increase over her 54 calories basal metabolism.

From these figures it is seen that, although the obese subject utilizes the 1,000 calorie no more economically than does the normal subject so far as her response to the specific dynamic action of the food is concerned, the 22 per cent increase above her basal metabolism has the appearance of a decrease in rate as compared to that of the 37 per cent increase above the basal metabolism of the normal subject.

This difference in percentages is due solely to the fact that the percentage caloric loss, due to the specific dynamic action of the food, is compared in the one case with a large caloric output of a large body (94 calories) and in the other case, with a relatively small caloric output from a smaller body (54 calories). Thus her apparently diminished response to the specific dynamic action of food in the obese subject is due to the larger size of her body and not to her obesity.

Assuming that the specific dynamic action of food is less in the obese than in the normal person is simply another way of saying that if the obese person eats as much as the normal person he will be overfed, because of his more economical use of calories which the normal person wastes through a more vigorous response to the specific dynamic action of food. Then, if through this more economical use of his calories the obese person becomes overfed, the same instincts which prompt the normal person to take less food after he has been overfed, and thus avoid gain in weight, should also prompt the obese person to take less food and thus also avoid gain in weight. Therefore, the reduced specific dynamic action theory leads one no nearer the solution of the problem of obesity, because it does not explain why the obese person does not instinctively reduce his food intake when overfed as does the normal person.

According to another theory, obesity is practically synonymous with hypopituitarism, and a large percentage of fat persons, whether sick or well, are diagnosed as cases of hypopituitarism and treated with pituitary extract or with dangerous doses of thyroid extract.

How much the failure of development of the testicles influences the development and function of the pituitary, and how much faulty development of or malignant growth in or about the pituitary body influences the growth and function of the testicles, is mere speculation that has so far led to nothing of practical use in treatment.

Late appearance of the various centers of ossification, and delayed union of the epiphyses, as shown by the x-ray, is seen in both eunuchoidism and hypopituitarism. Tandler and Groz called attention to this years ago.

Outwardly, also, the eunuchoid and the hypopituitary patient look much alike in facial expression, retarded sexual development, and in the more or less characteristic distribution of adipose tissue.

These two conditions represent *two* types of the so-called Fröhlich's syndrome, or dystrophia adiposogenitalis.

The Fröhlich's syndrome of hypopituitarism has its seat of pathology in or about the pituitary, is usually malignant, becomes progressively worse, affects the general health, dwarfs the mental and physical development, and leads to obesity as the result of a reduced basal metabolism as well as a reduced general energy output caused by the stunted mental and physical life.

The Fröhlich's syndrome of eunuchoidism has its seat of pathology in the genitals, is not malignant, often progressively improves during adolescence, does not affect the general health, does not affect the mental or physical features except as related to the secondary sexual characteristics, and leads to obesity, not as a result of a reduced basal metabolism, but as a result of a reduced total energy output, in other words, to habitual laziness, characteristic of individuals who fail to develop the normal male sexual characteristics, as seen, for example, in castrated males of any species.

Obesity and the associated dysmenorrhea or amenorrhea are no basis whatever for making the wholesale diagnosis of hypopituitarism, so prevalent in certain sections of this country today.

One of our leading medical journals, in a recent editorial, questions the validity of the law of conservation of energy as applied to the problem of obesity. It flatly maintains that "the problem of gain in weight is not so simple as it is pictured." It says: "Experience tends to lead one to question whether the body always responds with such a nicety of regulation." This editorial also observes that "certain persons remain lean despite a liberal appetite and unimpaired digestion, whereas others deposit fat with apparent ease." And finally the editor asks: "Has not the time arrived for considering obesity as a scientific problem, and talking about it less glibly?"

According to this editorial and to widespread belief, two persons, one fat the other thin, may eat at the same table. The fat one on a restricted diet gains weight, while the thin one on a generous food intake remains thin. The fat one works hard all day, the thin one is continuously idle, but still the fat one grows more fat, and the thin one more thin.

Similar to this are the many arguments offered by those who disbelieve in the principle of balancing energy intake against energy outgo and who believe that their own personal observation of such cases is a better guide to truth than any theory.

The answer to all such argument is that even if it be not purposely misrepresented in any of its details, one is positively in no position to argue either side of the proposition until all of the calories of the food intake of both the fat and the thin person are known in *actual measured units,* and not referred to by such as the above inexact terms, "restricted diet" and "generous food intake."

Then the total calories of the energy outgo must be similarly actually measured and not referred to by such inexact terms as, "works hard all day," or, "is continuously idle."

In so doing, it will be found that the thin person, although continuously idle, wastes large quantities of his calories of energy through repeated emotional storms of either anger or laughter, or pleasurable excitement, or habitual nervous fidgetting or worry, all of which are known to raise the metabolic rate more than does relatively hard manual labor. Then, too, it will be found that the fat one, although hard at work all day, goes about his duties with a minimum of physical effort and reacts to the pains and pleasures of life with a minimum of emotional strain.

At any rate, when the various sources of energy intake and outgo are all accurately taken into account and balanced against each other, the above type of argument, in its absurd attempt to contradict the law of conservation of energy, is inevitably shown to be a very inexact and untruthful statement of the actual caloric intake and outgo.

As a matter of fact, there is no need to explain obesity on the basis of theory. The United States Government and many of our educational institutions as well as governments and other institutions abroad, by spending large sums of money on costly devices, and by bearing the expenses of long tedious experiments, have proved over and over again that the animal body transforms the latent chemical energy of food into equivalent quantities of work and heat energy, and that, when the intake of chemical energy over- or under-balances the output of work and heat energy, there is a corresponding gain or loss, respectively, in body weight of protein, or glycogen, or fat in the body of the test subject.

An enormous literature on the results and interpretation of their work has accumulated, but it seems that none of this has percolated to the casual public, and very little of it to the medical press.

The names of Rubner, Voit, Lusk, Benedict, Atwater, Rosa, DuBois and many others are associated with this work. To these men, obesity requires no theory for its explanation. The one single simple explanation which applies to each and every case of obesity is that the cause of gain in fat and muscle weight (not counting the water and salt content of such tissues), is *due to the increase of the caloric intake over the caloric output.*

This explanation applies in every instance, whether the gain be much or little, whether it be from pregnancy or the menopause, hypopituitarism, hypothyroidism, laziness, powerful gastric motility, weak inhibition, temperament, appetite, easy living, gluttony, good food, or any unusual change in either the basal metabolic output or the total general metabolism.

For example, in the two types of Fröhlich's syndrome, the eunuchoid type and the hypopituitary type, the basal metabolism in the eunuchoid is normal; in the hypopituitary type it is about 25 per cent subnormal. But in the eunuchoid type the obesity, if present, is due to laziness, i.e., a decreased energy expenditure or general metabolism, in spite of a normal food intake; in the hypopituitary type it is due both to laziness and subnormal *basal* metabolism, in spite of a normal food intake.

The hypothyroid patient becomes obese for the same reason; adynamia and subnormal basal metabolism, associated with an almost normal food intake.

A normal person, changing to conditions of living which make less strenuous demands for physical labor, or social activities, or are less stimulating to the emotional life, will in a few years time become obese, if the same food intake, which previously maintained him or her at normal weight, be continued.

Obesity is not always present in hypothyroidism or hypopituitarism, be-

DISTRIBUTION OF A 3,000 CALORIE RATION

INTAKE IN CALORIES		OUTPUT IN CALORIES	
Intake	3,000	*Radiant energy:* maintains body temperature, minimum output of normal, fasting, waking, resting body, the basal metabolism. Varies with age, sex, indoor or outdoor life, thyroid medication, disturbances of emotional life, and in some diseases, hypo- and hyper-thyroid and pituitary conditions. (Medication with testicular, ovarian, or pituitary extract has no effect. Adrenalin, only slight and transient, i. e., about 20 minutes.) Calories	1,500
Water, inorganic salts and vitamins, although necessary to life, posses no chemical energy.			
Sufficient protein must be taken to maintain the body's nitrogen losses incident to cell repair, and secretion of mucus.		*Mechanical energy:* supports muscular contractions. Varies with kind of work; also in overfeeding, by increasing desire for exercise, and in underfeeding (to lose weight) by decreasing desire for exercise. Calories	500
Water is retained in tissues, in starvation, sometimes even more than enough to offset lost weight from fat loss, but this soon reaches its limit, and shows as edema when water of body reaches from two to eight pounds above its normal water content.		Lost as unabsorbed bits of food in feces. Varies with excess fat consumption, but may be measured and subtracted from intake. (Balancing intake against outgo is made on basis of intake to tissues, not to intestinal tract.) Calories	100
		Lost as epidermis, sebaceous material, hair, mucus, oil from skin. Varies with climate, strenuous labor, hot baths. (Losses from hot baths may be enormous.) Calories	50
Normally, food intake varies with motor efficiency of the stomach. No amount of previously laid down fat reserves can protect against "hunger pains" from an empty stomach.		Specific dynamic action of food. Varies with kind and amount of food. Averages 10 per cent of total caloric intake. Lost as increased body heat. Calories	300
		Total	2,450
		Balance	550
	3,000		3,000
Obese subjects normally have large gastric capacity and shortened emptying time of stomach even with high concentration of fat foods in diet.		This balance of 550 caloric excess is laid down as protein for immediate structural uses, as in growth and repair, and as glycogen, and fat for storage purposes, the resulting increase in body weight depending on how the 550 cal. are distributed as protein, glycogen and fat. If the 550 cal. balance is a shortage, the shortage is taken from body's previously stored reserves, and the weight loss is in proportion.	

cause in some cases the food intake has been decreased in proportion to the decrease in the energy outgo.

The cause of obesity seldom shows itself in the form of gluttony. Thus a normal 175 pound person at the age of twenty-five, taking at each mealtime a single mouthful of bread and butter in excess of the body's daily needs, will attain a weight of approximately 350 pounds by the time he is forty years old, and it is certain that no one could detect any evidence of gluttony when comparing two diets, one with and the other without the additional mouthful of bread and butter. Indeed it is of common observation that obesity is usually of extremely insidious onset.

The maintenance of a constant body weight under marked variation in bodily activity has also been the subject of frequent comment and wonderment.

The remarkableness of the phenomenon has been overrated. In a normal person, a gain in weight of five pounds of fat, when compared to the total body weight, it is true, is a small variation, but when compared to the three or four pounds of storage material already laid down, it is an enormous variation in gain of storage material. The accumulation of storage material is indeed about the least constant of the body's physiologic quantities. If wider variations in fat accumulations served a definite purpose in man's existence, as in the case of the hibernating bear, or the domestic hog, they would perhaps be in even greater evidence.

SUMMARY

1. The obesity of laziness and big eaters (simple obesity) and the obesity of eunuchoidism show no change in the basal metabolic rate, whereas the obesity of hypothyroidism, in both cretinism and myxedema, and the obesity of hypopituitarism, are associated with appreciable decreases in the basal metabolic rate.

2. Obesity is due to intake of food energy in excess of outgo of work and heat energy. Many factors influence the food intake. Similarly, many other factors influence the energy outgo. But no case of obesity can remain a mystery as to its cause if these factors have each been taken into consideration when balancing the total of the energy intake against the energy outgo.

3. In habitual undernutrition, the extreme opposite of obesity, the basal metabolism is markedly reduced. Reduction in the basal metabolism is, therefore, by no means necessarily associated with or followed by obesity.

4. The specific dynamic action of food, when calculated on the basis of the caloric intake, is not lower in the obese than in normals.

5. Many cases of obesity caused by a diet in excess of the normal body's needs (simple obesity) are often incorrectly diagnosed either as hypopituitarism or as hypothyroidism. Many cases of obesity due to eunuchoidism are also incorrectly diagnosed as cases of hypopituitarism.

6. Thyroid extract should never be used in the treatment of any form

of obesity in amounts sufficient to raise the basal metabolic rate to more than about 10 per cent plus.

7. Any attempt at reducing the body's energy intake of food, as in "dieting," is promptly compensated by the body's reduction in energy outgo, thus defeating the purpose of the dietary reduction, and too often effectively discouraging the patient with the "dieting" method of treatment for obesity.

SARCINAE IN PSORIASIS*

By Mary A. Marcus, A.B., M.S., Philadelphia, Pa.

REVIEWING the literature from an etiologic point of view, I was much impressed with the prevailing opinions as to the possibility of a parasitic nature of psoriasis.

The parasitic theory of psoriasis is strongly suggested from many points of view. Various organisms including fungi have been reported from time to time since 1853 when Wertheim[1] recovered a Penicillium from the urine of a psoriasis subject; later, 1879, Lang;[2] 1883, Eklund;[3] and, 1884, Wolff[4] also described mycelian fungi found in a lesion. These were later rejected by Neisser[5] and Reis[6] who showed that the alleged fungus was an artificial product of eleidin; in 1887 DeMattei[7] described a micrococcus, which he believed to be the causative agent, and produced psoriasis-like excrescences in inoculated animals, but this was regarded as an accidental finding by Majocchi.[7] In 1910 Sellei[8] described circular or ovoid, sharply outlined bodies found deep in the lesions in a case of psoriasis, and he regarded these bodies as the probable exciting cause. Crocker[5] also reported similar bodies, but their significance was not established. Pollitzer[9] and Haslund[10] believe that psoriasis is most probably due to an external microbic infectious agent; Mapather[11] is of the same opinion, but he adds that this microbic agent is an anaerobic one; Schamberg, Kolmer, Raiziss and Ringer,†[12] however, in their exhaustive study of the question of parasitism in psoriasis, arrived at no definite conclusion as to the parasitic cause of the disease. In this study they also reported an unidentified diplococcus, "X," isolated from five psoriatic lesions and from one blood culture; an ultramicroscopic, mobile, bacillar body demonstrated in seventeen out of nineteen psoriatics. Ultramicroscopic organisms resembling Paschen corpuscles of variola were reported by Sercowski and Wisnewski.[28]

More recently, 1919, Bory[13] also reports small, irregular, rod-like germs resembling mycelian threads which he found repeatedly in psoriasis lesions.

Many attempts to transmit the disease by direct inoculations have failed with the exception of two instances. In 1885, Lassar[14] was apparently able to show the transmissibility of psoriasis from man to rabbits. A rabbit was inoculated with blood lymph and psoriatic scales from a human subject, and with

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Read before the Society of Clinical Pathologists, May 20 to 23, 1925.

†These admirable studies should be consulted by all future investigators interested in the etiology of psoriasis.

similar material from this rabbit a second rabbit was inoculated and produced lesions suggestive of psoriasis. Behrend did not believe that these lesions conformed with the lesions in the human psoriasis, but according to his opinion they resembled tonsurans lesions.[21] Neisser,[5] Rindfleisch, Majocchi[7] and others failed to corroborate Lang. In 1887 Tenholt[15] ascertained that several individuals of a family contracted the disease from a dutch beef. The nurse in turn contracted the disease from these patients, but this malady could not be transmitted to the animal from the human subjects. Destot,[16] however, was apparently successfully inoculated from an infant who had vaccinal psoriasis; Ducrey,[17] DeAmicia,[5] Alibery,[5] and Hammer and Block[5] in a long series of inoculations, using men, rabbits, guinea pigs, and dogs, were unable to obtain any but negative results; Wutzdorff's and Schamberg's[18] efforts to inoculate themselves were also unsuccessful.

It is worth recalling that, in the above-mentioned studies made by Schamberg and his associates[12] in which antigen employed for syphilis was used, nine suggestive complement-fixation reactions, not accounted for by other evidences of syphilis, were obtained from 48 psoriatic subjects; and that some 16 different organisms, isolated from cases of psoriasis, were studied with the conclusion that there was lack of causal relation to the disease.

It seems to date that none of the foregoing findings have been either generally accepted or, in fact, sufficiently confirmed to lend even a strong suspicion for their specific pathogenicity of psoriasis.

The parasitic theory of psoriasis is further suggested by the fact that psoriasis follows vaccinations,[19] attacks of tonsillitis,[20] acute toxemias,[21] and various injuries, and by the fact that the border of one patch melts away when in contact with that of another.[22, 24] The disease also seems in some stages to be influenced favorably by exposure to the actinic rays of the sun. The distribution of the psoriatic lesions also suggests that the disease may be influenced by sunlight; Schamberg,[22] Knowles,[23] Stelwagon[24] and Ormsby[25] believe that the disease is much more prevalent in the winter months and in cold countries than in warm seasons and climates. Kayser[26] reported that in the tropics psoriasis is rare, and the symptoms of the disease, when they do develop, are rudimentary, the typical eruption scarcely ever being produced.

Sutton and others[27] believe that eradication of focal infection leads to prompter disappearance of the psoriatic eruption under the usual treatment.

In the bacteriologic study of the skin the technical difficulties are great. It has been noted from the literature that the predominating findings thus far obtained appear to be saprophytes, normally found in the skin. It is thus evident that in the study of a skin disease, such as psoriasis there are many complications incident to contamination by saprophytes; conclusions, therefore, are unreliable unless this contamination is reduced to a minimum. The following method was devised with this end in view.

METHOD OF STUDY

Materials were selected routinely from three different levels in such lesions, both for smears and cultures.

(1) The lesion was washed with 70 per cent and 95 per cent alcohol, and some of the scales were at once transferred to the culture media*; others were kept for an hour in 60 per cent alcohol and thereafter inoculated upon the culture media.

(2) After the scales had been removed, the second level was sterilized with 70 per cent and 95 per cent alcohol and irritated in order to provoke oozing of enough serum (lymph) to inoculate a culture media and for the preparation of impression smears and smears of mixed serum and scales.

(3) In making the third series of inoculations the exposed surface was again sterilized with alcohol, further irritated, and, from the renewed oozing, serum cultures and smears were prepared as before.

Three or not less than two lesions were thus treated; an average of ten inoculations and as many smears represented the extent to which the material was examined bacteriologically in each case.

(4) For taking blood cultures the surface of the forearm was sterilized as follows: The surface was washed with soap and water, alcohol and ether, and followed by mercurochrome 1:1000 in 50 per cent alcohol, and covered with dressing for a half hour.

The reason for the care in sterilizing the tissues prior to taking materials for cultures is that thereby one reduces the liability for surface contamination, if present, to intermix with the deeper causative agents.

(5) All smear preparations, made both from the cultures and directly from the lesions, were fixed (after drying in the air) by flame and in a 50:50 mixture of ether and alcohol. They were stained by Gram's, Muir's, Rosenow's capsule, and Giemsa's methods, respectively. Giemsa stain has proved equally good either in applying the stain directly on the smear for fifteen to fifty minutes or in staining them overnight and then washing them with the different solutions of acetone and xylol, as is the procedure for the tissue specimens. Muir's capsule method has proved very satisfactory for demonstrating the capsule of these sarcinae. (The carbolfuchsin was applied cold for an hour.)

RESULTS

Materials for this bacteriologic study were obtained from forty-one psoriatic subjects and from sixteen patients of other dermatoses.

In the examination of the lesions and blood in the forty psoriatic patients, thirty-eight (95 per cent) showed apparently identical sarcinae, and the remaining two (5 per cent) were negative. Five of the forty also showed staphylococci (12.5 per cent); three diplococci (7.5 per cent); two B. subtilus (5 per cent), and two B. pseudodiphtheria (5 per cent).

The same sarcinae (microscopically) have been demonstrated, so far, in fifteen smears made of the oozing serum and scales from the lesion, in eight smears of the scales and serum, in six of scales only, incubated (upon the same culture media given above) for seventy-two to ninety-six hours at 37° C., and in an impression smear made of the serum. Fifteen different psoriatic patients were thus examined.

*The culture media employed consisted of plain agar, glucose agar, maltose agar, Sabouround's and all other ordinary media.

Upon examination of the sixteen different controls, selected from other skin diseases, including eight cases of tinea circinata, three of pityriasis rosea, two of lichen planus, one of Duhring's disease, one of secondary syphilis and one of pellagra, a sarcinae was encountered in but one, that of pellagra. This organism lost its morphology in subcultures and reduced none of the sugars, which were affected by the sarcinae recovered from psoriasis. These atypical sarcinae were, therefore, excluded from further comparative study, especially since the usual method of the skin disinfection had not been followed. Ten additional cultures from normal skin areas of psoriatic subjects showed no sarcinae.

Blood cultures were made from three psoriatic patients, and from one of these (an acute case of two weeks' duration), similar sarcinae were obtained, but none from the two others (one three years' and the other of twenty years' duration).

Histologic sections made of the lesions of five psoratic subjects have thus far been carefully examined. Various staining methods were used which will be described in detail in the next paper. In two sections from two different subjects, stained by Muir's method, the sarcinae were successfully demonstrated. In each case the sarcinae were found in the epiderm, once near the scaling surface, the other time fairly deep in the epithelial layers and in the exudate.

In order to determine whether or not the sarcinae obtained from the psoriatic subjects were of the same species, further cultural and microscopic studies were made as follows:

CULTURAL CHARACTERISTICS

The primary growths of the sarcinae appeared to be invariably either large (3 by 5 mm.) elevated plate-like strata or very inconspicuous colonies on the media thus far employed. Often they were concealed in some of the blood clot transferred to the surface of the media and were apt to be overlooked and lost. The size of a single colony rarely exceeded 3 to 5 mm. in diameter. In the older examples and in the subcultures upon the agar media were usually very fine, raised, pearly, glistening colonies of dry, thick, plate-like growths of grayish-yellow or milky-gray color. Broth transplants were usually clouded with an ultimate production of a gray or grayish-yellow pellicle or sediment. In milk, an acid reaction usually occurred accompanied by coagulation (in only three-instances the milk was rendered alkaline and no coagulation followed). Subcultures on potato were often invisible grossly, but again on the same raw potato there appeared heavy grayish-yellow or copper-orange discrete colonies. The Diffco potato almost without exception gave rise to fine, round, raised, grayish-yellow to orange colonies.

Most of the thirty-eight cultures from psoriatic lesions produced acid in the ordinary sugar media, including dextrose, lactose, maltose, saccharose, levulose, mannite, dextrin, Russell double sugar and inulin. They reduced nitrates to nitrites, and occasionally liquefied gelatine. The biologic property of reducing sugars was, however, usually lost by old cultures and the growth was markedly retarded. It was observed that light inhibited growth and its absence hastened the growth, but with differences in the development. In one

Bergey, Table I	Marcus, Table II
1. Division occurs under favorable conditions in three planes.	Same division.
2. Producing regular packets.	Producing regular and irregular packets of 2, 4, 8, 16, 32 and larger groups.
3. Growth on agar abundant, usually yellow, orange pigment.	Pigment usually whitish-gray or grayish-yellow.
4. Dextrose broth slightly acid, lactose generally neutral.	Dextrose, lactose, maltose, saccharose, dextrin, mannite, inulin and Russell double sugar markedly acid.
5. Gelatin frequently liquified.	Gelatin rarely liquified.
6. Nitrates may or may not be reduced to nitrites.	Nitrates are usually reduced to nitrites.
7. No capsule reported.	Capsule demonstrated.

Table III

Sugar Reactions Produced by the Sarcinae Recovered from Psoriasis

No. of Patients	No. of Litmus Agar				Carbohydrates				Neutral Red Bouillon			
	Dextrose	Lactose	Maltose	Saccharose	Russell Double Sugar	Mannite	Dextrin	Triple S.	Inulin	Dextrose	Lactose	Maltose
1												
2	A	A	A	A	A	A	A	A	A	A	A	A
3												
4	O	O	A	A	ALK	O	O	?	A	A	A	A
5	A	A	A	A		O	O	O	?	A	A	A
6	O	?	A	?	ALK	O	O	?	?	A	A	A
7												
8	A	A	A	A	O	O	?	?		A	A	A
9	A	A	A	A	A	A	A	A	A	A	A	A
10	A	A	A	A	A	A	A	A		A	A	A
11	A	A	A	A	A				A	A	A	A
12	A	A	A	A	A	A	A	A		A	A	A
13	O	A	A	A	O	?	?	A	A	A	A	?
14	A	A	A	A	A	A	A	A	A	A	A	A
15												
16	A	A	A	A	A	A	A	A	A	A	A	A
17	A	A	A	A	A	A	A	A		A	A	A
18	A	A	A	A	A	A	A	A		A	A	A
19	O	A	O	O	O	O	O	A	O	A	A	A
20	O	O		A	O	O	A	O	O	A	A	A
21												
22	A	A	A	A	A	A	A	A	A	A	A	A
23												
24	O	O	A	A	ALK	O	O	?	A	A	A	A
25	A	A	A	A		O	O	O	?	A	A	A
26	O	?	A	?	ALK	O	O	?	?	A	A	A
27												
28	A	A	A	A	O	O	?	?	A	A	A	A
29	A	A	A	A	A	A	A	A	A	A	A	A
30	A	A	A	A	A	A	A	A	A	A	A	A
31	A	A	A	A	A	A	A	A	A	A	A	A
32	A	A	A	A	A	A	A	A	A	A	A	A
33	O	A	A	A	O	?	?	A	A	A	A	?
34	A	A	A	A	A	A	A	A	A	A	A	A
35												
36	A	A	A	A	A	A	A	A	A	A	A	A
37	A	A	A	A	A	A	A	A	A	A	A	A
38	A	A	A	A	A	A	A	A	A	A	A	A
39	O	A	O	O	O	O	O	A	O	A	A	A
40	O	O	A	A	O	O	A	O	O	A	A	A

A—acid; Alk—alkaline; O—neutral.

TABLE IV

SOME SPECIAL BIOLOGIC FEATURES OF THE SARCINAE RECOVERED FROM PSORIASIS

Patient's No.	666				LITMUS MILK DAYS						NITRATES REDUCED NITRITES DAYS					THERMAL DEATH POINT WITH N/10 VOLERIC ACID			HEAT ONLY					
	Yellowish-Gray	Milky-Gray	Citro-Yellow	Milk-Coagul.	1	2	4	6	8	10	Indol Product.	2	4	6	8	Without Acid min.	Degrees of Temperature	Time—Minutes with N/10 Acid	Opt. Temp.	Capsule	Motility	Gelat. Liquef.	Gram's Stain.	
1		+		+	A	A	A	A	A		+	+	+	+		20	80° C.	10	37°-40° C.		−	±	+	
2	+	+		−	A	A	A	A	A		+	+	+	+		"	"	"	37°-40° C.	+	−	+	+	
3		+		+	A	A	A	A	A		−	−	−	−		"	"	"	" " "	+	−	−	+	
4		+		+	O	O	A	A	A		−	−	−	−		"	"	"	" " "	+	−	−	+	
5		+		−	A	A	A	A	A		−	+	+	+		"	"	"	35°-40° C.	+	−	+	+	
6	+	+		+	O	O	O	O	O		−	−	−	−		"	"	"	35°-37° C.	+	−	−	+	
7	−	+														"	"	"	30°-35° C.	+		−	−	
8	+	+		−	A	A	A	A	A		+	+	+	+		"	"	"	35°-38° C.	+	−	+	+	
9	+	+		−	A	A	A	ALK	ALK		−	−	−	−		"	"	"	37°-40° C.	+	−	−	+	
10	+		+	+	A	A	A	A	A		−	+	+	+		"	"	"	25°-35° C.	+	+	+	+	
11	+				O	O	O	O	O			O	O	O		"	"	"	25°-35° C.	+	−	−	−	
12	−																							
13	+	+		+	A	A	A	A	A		+	+	+	+		"	"	"	35°-38° C.	+	−	+	+	
14	+		−	+	A	A	A	A	A		−	−	−	−		"	"	"	" " "	+	−	+	+	
15	+	+		+	A	A	A	ALK	ALK		−	−	−	−		"	"	"	" 40° C.	+	−	−	+	
16	+			−	A	A	A	A	A		+	−	+	+		"	"	"	" " "	+	−	+	+	
17	+	+		−	A	A	A	A	A		−	−	−	−		"	"	"	" " "	+	−	−	+	
18	+	+		−	A	A	A	A	A		−	−	−	−		"	"	"	" " "	−	+	−	+	
19	+	+			O	O	O	O	O		−	−	−	−		"	"	"	" " "	−	−	−	−	
20	−			−	O	O	O	O	O		−	−	−	−		"	"	"	37°-40° C.	+	+	+	+	
21	−	+		−	O	O	O	O	O		+	−	−	+		"	"	"	" " "	+	+	−	+	
22	+	+		+	ALK	ALK	ALK	A	A		+	−	−	+		"	"	"	" " "	−	−	+	+	
23	−			−	A	A	A	A	A		−	−	−	−		"	"	"	" " "	+	−	+	+	
24	−	+		+	A	A	A	A	A		−	−	−	−		"	"	"	" " "	−	+	−	+	

TABLE IV—CONT'D

Patient's No.	666 Yellowish-Gray	666 Milky-Gray	666 Citro-Yellow	666 Milk-Coagul.	Litmus Milk Days 1	2	4	6	8	10	Nitrates Reduced / Nitrites Days: Indol Product.	2	4	6	8	Thermal Death Point with N/10 — Voleric Acid: Without Acid min.	Degrees of Temperature	Time—Minutes with N/10 Acid	Heat Only: Opt. Temp.	Capsule	Motility	Gelat. Liquef.	Gram's Stain.
25	–	+		–	A	A	A	A	A		–	–	–	+		20	80° C.	10	37°-40° C.	+	–	–	+
26	+	+		–	A	A	A	A	A		–	–	–	–		"	"	"	" " "	+	–	–	–
27																				–			–
28	+	+		–	A	A	A	A	A		+	+	+	+		"	"	"	" " "	+	–	–	+
29	–	+		–	O	O	ALK	ALK	ALK		–	–	–	+		"	"	"	" " "	–	–	+	–
30	+		+	+	A	A	A	A	A		–	–	–	–		"	"	"	" " "	+	–	–	+
31	–	+	–	–	A	A	A	A	A		+	–	–	–		"	"	"	" " "	+	–	–	+
32																				–	–	–	–
33	+		+	–	O	O	O	O	O		–	–	–	–		"	"	"	" " "	+	–	+	+
34		+		+	A	A	A	A	A		–	–	–	–		"	"	"	" " "	–	–	–	–
35		+		–	A	A	A	A	A		+	+	+	+		"	"	"	" " "	+	–	–	+
36	+			?	?	?	?	ALK	ALK		–	–	–	–		"	"	"	" " "	+	–	–	+
37	+	+		–	A	A	A	A	A		–	–	–	–		"	"	"	" " "	+	–	–	+
38		+	–	–	O	ALK	ALK	A	A		+	+	+	+		"	"	"	" " "	+	–	–	+
39		+		–	O	O	A	A	A		+	–	–	–		"	"	"	" " "	–	–	+	+
40	–	+		–	O	O	O	O	O		–	–	–	–		"	"	"	" " "	–	–	–	–
41																							
Total	20+	28+	3+	12+	23A 1ALK	23A 2ALK	24A 3ALK	25A 4ALK	25A 4ALK		12+	9+ –	10+	14+		38 in 20 min.	38 at 80° C.	38 in 10 min.	27 at 37°-40° C. 3 at 35-38 6 35-40	28+	4+ 6	14+ –	30+ –

SUMMARY OF TABLES NOS. III AND IV

Initial growth with thick elevated strata in cultures—1, 2, 3, 4, 5, 6, 9, 13, 19, 20, 22, 22	(12)
Initial growth with plate-like strata in cultures—1, 3, 5, 6, 23	(5)
Initial growth with elev. large moist opaque col. cultures—9, 15, 18, 22	(4)
Initial growth with med. dry glistening col. cultures—9, 10, 14, 22, 23, 24, 25, 26	(8)
Initial growth with fine pin-point dry col. cultures—9, 10, 21, 23	(4)
Subcultures with elev. large dry porcelain-white col. on maltose agar—2, 5, 22, 23, 24	(5)
Subcultures with elev. large moist yellowish-gray col. on maltose agar—9, 10, 15, 18	(4)
Subcultures with elev. large thick moist yellowish-gray surface growth on all agar sugars—9, 10, 15, 18	(4)
Subcultures with very fine granular almost invisible surface growth on all culture media including all other strains—	(33)
Subcultures with acid production on glucose—	(20)
Subcultures with acid production on lactose—	(26)
Subcultures with acid production on maltose—	(28)
Subcultures with acid production on maltose neutral red—	(30)
Subcultures with acid production on levulose—	(17)
Subcultures with acid production on saccharose—	(33)
Subcultures with acid production on Russell double sugar—	(15)
Subcultures with alkal. production on Russell double sugar—	(6)
Subcultures with acid production on mannite—	(16)
Subcultures with acid production on dextrin—	(19)
Subcultures with acid production on dextrose neutral red—	(39)
Subcultures with acid production on triple sugar—	(21)
Subcultures with acid production on inulin—	(17)
Subcultures with acid production on litmus milk—	(24)
Subcultures with alkal. production on litmus milk following acid reaction—	(2)
Subcultures with alkal. production on litmus milk preceding acid reaction—	(4)
Subcultures with reduction of nitrates to nitrites—	(14)

instance a culture which had appeared dead for about eight months, spontaneously began to show a secondary growth superficial to the old one, after it had been stored away from the direct sunlight for three months. This secondary growth appeared heavier, creamier, and more active than the ordinary culture, which was still inert.

It is therefore probable that dissociation of growth and the other biologic properties of the sarcinae are caused by both age and light.

MICROSCOPIC APPEARANCE

Microscopically the sarcinae, both in the initial and in as many as eight generations of subcultures, appeared as spheres averaging from 1.5 to 3.5 microns, arranged as pairs or in packets of 4, 8, 16, or even in groups larger than 32 or 64. Although the individuals in such packets were usually all equal in size, packets of eight spheres with one or more of the constituent spheres notably larger or smaller than the rest, thus deforming the usually regular cubes, were not infrequently found. When cubes stained by Gram's method were stained by Muir's method, either the cube or the individual coccus showed a distinct capsule.

The sarcinae are believed to be identical in all thirty-eight cultures, showing similar cultural and microscopic characteristics, but possessing distinct variations, amounting almost to pleomorphism,—a feature not uncommon in other known species of sarcinae. That these variations in the size of the individual spheres and packets, in pigmentation, in behavior in milk, in sugar media and liquefaction in gelatine are not specific differences is shown by the fact that such variations occurred occasionally with organisms derived from some

single colony, and that reversal of the variations were at times obtained. The details are given in Tables Nos. III, IV, and V.

HUMAN INOCULATIONS

Normal skin areas of twelve psoriatic subjects and one normal human volunteer were scarified and inoculated with pure cultures of the sarcinae isolated from psoriatic lesions; as a control a sterile 0.85 per cent salt solution was used. The inoculations were made about one inch apart. In a control of normal saline an erythema developed. At the site of the sarcinae injections there developed six out of twelve positive reactions. Three reactions following the sarcinae inoculation showed not only erythema and infiltration, but also a fine branny scaling and three weeks later, a white ring (suggesting that the tissue had been damaged and was not yet healed) around the infiltrated reddish center. One showed a very pronounced course with silvery scaling; in the other two only an erythema similar to that in the control appeared. Whether or not this is a specific action of the parasitic sarcinae or simply the result of mechanical irritation, as noted in certain cases by Schamberg, cannot yet be determined. The six psoriatic subjects, in whom the sarcinae inoculations were productive of reactions, included two subacute cases of about one year's duration each, two acute cases of two weeks' and two months' duration and two chronic cases with a history of three and twenty years, respectively.

ANIMAL INOCULATIONS

Experiments were conducted on one monkey and two rabbits; the monkey received five subcutaneous injections of 1 c.c. each of sarcinae suspensions, together with five scarification inoculations simultaneously on ten different areas of the skin; none of these as yet show any reaction. The same monkey and two rabbits received subcutaneously 0.5 c.c. sarcinae suspension into the outer side of the ear. A week later the areas inoculated of both species of animals were swollen and hard, and on excision showed a purulent discharge, from which by smear and culture the same type of sarcinae were demonstrated. No evidence of scaling, similar to that observed in psoriasis, had appeared during the one week interval prior to excision.

AGGLUTINATION AND COMPLEMENT FIXATION

If antibodies or agglutinins existed in the body fluids of psoriatic subjects in demonstrable amount, and if the antigen, presumably a parasite, were the sarcinae, this should yield with the antibody, complement fixation and agglutination, respectively.

SEROLOGIC TECHNIC

At the suggestions of Dr. J. A. Kolmer, several experiments were performed as follows:

Six different cultures of sarcinae isolated from the lesions of six psoriatic subjects were subcultured on agar, six times in succession and washed off with 0.3 per cent phenolated salt solution. The washings were shaken automatically for four hours and then heated to 60° C., for one and a half hours. Two mix-

tures of three pure sarcinae cultures each were prepared in a similar manner. Agglutination tests were performed with six of these antigens diluted 1:10, 1:20, and 1:40, respectively, against five psoriatic sera, one syphilitic serum, and one normal control serum; none showed agglutination.

Four of the same antigens and four psoriatic sera were employed for the complement-fixation tests* according to Kolmer's[31] new method. The results were all negative. Further research in serology is therefore projected.

DISCUSSION

In comparing the sarcinae isolated from the psoriatic lesion and the one blood culture, with Bergey's tabulation of the group of sarcinae, it is evident that the organism recovered belongs to Genus VII sarcinae (Goodsir).[29] Winslow and Rogers described it as follows: "Saprophytes and facultative parasites. Division occurs under favorable conditions in three planes, producing regular packets. Usually gram-positive. Growth on agar abundant, usually with formation of yellow or orange pigment. Dextrose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied. Nitrates may or may not be reduced."

The essential differences of the sarcinae classified by Bergey[30] and the sarcinae isolated from psoriasis are shown in (Bergey's and in the writer's) Tables I and II.

It is a well-known fact that by the application of the actinic rays (when the psoriatic eruption is quiescent) the subject is often relieved of the psoriatic lesions; that the disease is influenced by sunlight is noted from the fact that the eruption usually occurs on the parts of the body covered by clothing, such as the elbows, knees, thorax, abdomen, and sacrolumbar regions. And further, the disease is worse in the winter and in cold climates, being better, disappearing or rarely occurring in the summer and in warm climates. It seems coincidental that the growth of the sarcinae, isolated from the psoriatic subjects, is inhibited by sunlight and thereby its biologic properties to reduce sugars are lost. (In the dark, at room temperature, the growth was observed to be active, but it was inhibited at room temperature in the presence of diffuse daylight, and, when the cultures were kept thus for ten weeks in the diffuse daylight, they would give no growth in repeated subcultures.) Active growth recurred after the old culture had been stored away from direct sunlight for three months and after eight subcultures had been made. A new growth of thick, creamy, heavy masses, superficial to the old dead surface growth occurred spontaneously, the old cultures being dead for about eight months.

A word may be said in favor of the technic devised for this study. In the forty-one subjects of psoriasis from which fifty cultures of bacteria have been obtained, only five showed staphylococcus, which is known to be the most common inhabitant of the skin, and only 5 per cent of diphtheroids, which is found in about 15 per cent of all skin cases.

*I am indebted to Miss Carola Richter of the Research Institute of Cutaneous Medicine, Philadelphia, for her kind assistance in the complement-fixation work.

Diphtheroids found in two blood cultures were reported by Schamberg, Kolmer and their associates. They also reported five other species of bacilli and ten different species of cocci, recovered from the lesions, which included a diplococcus "X" isolated from five lesions and one blood culture. All of these sixteen different species were based on the study of twenty-four psoriatic cases from which fifty-seven cultures have been obtained, but no sarcinae were reported.

In the present study of the forty psoriatic cases from which fifty cultures have been obtained, only five different species of bacteria were isolated, which included thirty-eight cultures (95 per cent) of one species of sarcinae isolated from the lesions and one blood culture, five cultures (12.5 per cent) of staphylococci, only about 7.5 per cent of diplococci, and but 5 per cent, two cultures each of B. subtilis and B. pseudodiphtheria isolated from the lesion only. In some instances sarcinae were demonstrated in the direct smear, while the cultures of the same case were negative. It would suggest that the organism was killed either by sterilization of the skin with alcohol or by treatment.

Thus it is readily seen that the technic used in this study largely obviates contamination.

In every case the cultures confirmed the findings of sarcinae in the fifteen direct smears, so far examined. Eight of the fifteen were made from the scales and oozing serum of the lesions, one impression smear made only of the serum (by pressing the slide upon the irritated surface of the lesion); six smears were made of scale and incubated at 37° C. for seventy-two to ninety-six hours. Sarcinae were also found in two histologic sections and in the one blood culture. It is worth recalling that the two sections showed the sarcinae not only in the epiderm but also once deep in the epithelial layers as well as in the cellular exudate.

Although no typical sarcinae were found in the fifteen control cases, selected from other skin diseases, and none were obtained in the additional control cultures made from normal skin areas of ten psoriatic subjects, our studies are being continued.

Even though six out of twelve sarcinae inoculations, by scarification in psoriatic patients, were productive of psoriatic lesions, it is unwarranted at the present time to assert the parasitic nature of the sarcinae to psoriasis, as one of the controls gave a similar reaction to that observed in the two last sarcinae inoculations. Therefore, whether the causative agent of the disease in question has or has not been isolated, only time and further study can tell. It is interesting to note, however, that the sarcinae here described have been isolated from the lesions studied in a proportion as high as 95 per cent.

Further attempts at serologic study, vaccine treatment and inoculations to reproduce the disease by different methods are being made. Especially the study to relate the histologic changes of the lesions with demonstrable sarcinae in all the psoriatic subjects studied bacteriologically must be completed in order to arrive at a definite conclusion as to the significance of the sarcinae found.

SUMMARY

1. A survey of the literature on the parasitology of psoriasis showed that most of the data thus far recorded appeared to be by the commoner saprophytes of the skin.

2. In order to overcome this contamination, a special method was devised.

3. The efficiency of this technic is shown by the fact that in 50 cultures from 40 psoriatics there were only 5 cultures (12.5 per cent) of staphylococci, 2 cultures each (5 per cent) of B. subtilis and B. pseudodiphtheria, and 3 cultures (7.5 per cent) of diplococci.

4. From the 50 cultures of 40 psoriatics, 38 strains (95 per cent) of a sarcinae apparently of identical species were obtained.

5. Sarcinae of the same morphology were demonstrated in 15 direct smears (15 patients) and also in histologic sections from two different patients.

6. Similar organisms were not recovered in cultures and smears of 16 controls (sundry scaly dermatoses), nor from the sound skin of 10 psoriatic subjects.

7. Six agglutination and four complement-fixation tests gave negative results.

8. Attempts to reproduce psoriatic lesions by inoculations into man and animals are in course and will be reported in detail in a later communication.

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DISCUSSION

Dr. Francis L. Burnett.—I have been interested in Miss Marcus' paper, because I have also been making a study of psoriasis. This disorder of the skin is supposed to be either inflammatory or metabolic in origin. In consideration of the infectious theory about which you have already heard, I wish to say a few words for the metabolic hypothesis. In the studies I have made in nutrition I have considered food factors from the absorption of digested products as determined by the form of the feces and the intestinal rate. Complete and proper nourishment from this point of view is a delicate and complex process in which many food factors have to be adjusted and generally controlled. Many of the patients with psoriasis eat too fast and too much or badly proportioned food; and on this account there is malabsorption. The skin fails to get the digested products essential for normal maintenance. By dietary treatment, when the patient is educated to eat slowly, select properly proportioned food, etc., there is a change in the intestinal rate and the anabolic processes are improved. Under these circumstances the lesions of the skin gradually disappear; and, if the patient continues treatment and the elimination of the faulty food factors, another attack will be prevented. Accordingly, from the metabolic point of view we not only have a very logical basis for the treatment of the disease, but also a means of maintaining the health of the skin.

Dr. Martha A. Wood.—What the previous speaker has said corresponds with my experience. In one case a patient came for examination of feces. I found that the feces were loaded with trichomonas and a too frequent rate of stools during the day. He was put under treatment for the intestinal condition and was cured.

Dr. John A. Kolmer.—Owing to the wide prevalence of psoriasis and the fact that in some cases the clinical history suggests a possible parasitic origin, the work reported by Miss Marcus properly engages our attention. As correctly stated by her it is doubtful whether the disease has ever been certainly transmitted to a normal human being or lower animal, although it is a common clinical experience that new lesions may be produced in the psoriatic cutaneous irritation. The apparent impossibilty of transmitting the disease to the lower animals has naturally greatly added to difficulties in etiologic studies in this disease; in a general way it also detracts from its parasitic origin.

At the Research Institute for Cutaneous Diseases where Miss Marcus has done a part of her work, both Dr. Schamberg and I have been favorably impressed with the great care exercised by her in making cultures of the skin. Naturally this is a consideration of first rate importance, and errors doubtless account in large part for the numerous organisms described from time to time as the etiologic agents of psoriasis.

At the Institute we have been mostly impressed by the frequency with which Miss Marcus has found sarcinae in psoriasis and its infrequency in control cases, many of whom presented eczematous lesions favorable for bacterial growth. It is necessary, however, to reserve all opinion of the possible relationship of sarcinae to psoriasis and particularly since minor morphologic and cultural differences from other sarcinae are of very little importance. The work is, however, deserving of proper attention, and it is to be hoped that the investigation will be continued to final conclusions.

Dr. John A. Kolmer (again discussing Miss Marcus' paper).—I would sound a note of warning against hasty conclusions of the value of dietary or other forms of treatment in this disease. Dr. Schamberg, Dr. Raiziss, Dr. Ringer, and I found, some years ago, that psoriatics may show marked disturbances of nitrogen metabolism and that a reduced protein intake sometimes favorably influences the clinical cause of the disease, but it is characteristic of psoriasis that periods of temporary improvement are followed by exacerbations which may readily lead to erroneous conclusions regarding any particular method of treatment.

Miss Mary Marcus (closing).—I agree with Dr. Burnett when he says that there is an element of metabolism in the causation of psoriasis. There is no doubt that there is very frequently an improvement in the condition of the patient following the elimination of certain food factors. For that matter, the disease may often disappear temporarily without any change in the diet whatever. The question is not how we can treat the disease palliatively and temporarily, but how can it be cured effectively and permanently?

In reference to the remarks of Dr. Martha A. Wood, it is not quite clear to me when she says: "He (the patient) was put under treatment for the intestinal condition and was cured," whether she meant to infer that the intestinal condition was cured or that the psoriasis itself was cured. Even if the latter was meant, it would not at all prove anything, because of the fact that only one case was treated and even that case was not followed up.

What I am anxious to emphasize, however, is that after a great deal of work along these lines, treating the patients by inoculations with the pure cultures of sarcinae and the filamentous organism recovered from the lesion of psoriasis, I have succeeded in clearing up five cases. Of course, I am still continuing my experiments in a number of cases, and I intend to follow them all up to prove to my own satisfaction, as well as to the satisfaction of the profession, whether or not the results are going to be permanent.

In my report to the New York Dermatological Society on December 16, 1925, I have taken pains in stressing this, to me, very important question of the existence of the filamentous organism, associated with the sarcinae in psoriasis. These organisms I have repeatedly demonstrated bacteriologically in culture and in the tissue itself.

In conclusion, I wish to ask all the coworkers along these lines to be good enough to communicate with me in reference to any results they may have obtained or any information that they can possibly furnish me with reference to this work, in which I am extremely interested.

This is but a preliminary report and far from complete.

LABORATORY METHODS

THE RAPID DETERMINATION OF ALBUMIN IN URINE*

By F. B. Kingsbury, Ph.D., Charles P. Clark, M.D., Gertrude Williams, M.S., and Anna L. Post, A.B., New York City

THE need for an accurate, rapid method for determining albumin in the urine in the smaller amounts, that is, from 0.01 per cent to 0.1 per cent, is quite apparent to anyone who has had occasion to examine urinalysis reports. Heretofore the albumin estimation has been made by the Heller's ring test or by the heat and acetic acid coagulation test under conditions which were not necessarily optimum, and the results were recorded as "slight trace," "trace," "+," "++," etc. These terms are subject to great variation in meaning since they are based upon methods which are not quantitative and which give ample opportunity for great differences in interpretation between individual analysts.

In 1914 Folin and Denis[1] described a method for the quantitative determination of albumin in urine based on the turbidity produced by the precipitating action of sulphosalicylic acid on this substance and comparing a turbidity so produced with that made under the same conditions with a standard protein solution. In 1922 Folin[2] simplified the original method by carrying out the operation in graduated test tubes. Ten urines were treated with sulphosalicylic acid reagent simultaneously with the necessary number of albumin standards, 5 or 6, similarly treated. The estimations were then made by a side by side comparison of known with unknown.

This method was successfully used in this laboratory for more than a year, but it was seen that the repeated preparation of sets of tubes from the various serum protein standards for each ten or twenty urine specimens, involving as it did the obtaining and analyzing of sheep serum with its subsequent careful dilution, would prevent any extended application of this otherwise suitable method. On this account it was undertaken to devise standards which would be permanent with which to replace the freshly precipitated serum standards. The purpose of this paper is to describe the preparation and use of such standards and thus to make possible the extension of this valuable method.

During our work we found that the original dilution at which the turbidity was produced, that is, 1 c.c. of urine or albumin standard solution diluted to 25 c.c. with 2 per cent sulphosalicylic acid was less adequate in determining the lower levels of albumin, 0.01 to 0.05 per cent, than a dilution of 2.5 c.c. of urine to 10 c.c. with 3 per cent sulphosalicylic acid. The latter dilution was accordingly adopted in all the work reported in this paper.

*From the Biochemical Laboratories of the Metropolitan Life Insurance Company, New York.

Received for publication, November 24, 1925.

The first attempts to make permanent standards showed that it was desirable to use suspensions, the particles of which were nearly the same size as those causing the turbidity in the average albuminous urine.

For this reason we tried to use the albumin-sulphosalicylate dispersions themselves as permanent standards, but, since the particles of such suspensions always formed larger aggregates, thus materially changing the turbidimetric value of the standard even in the presence of protective colloids, this type of standard with water as the suspending medium had to be discarded. It was necessary, therefore, to use solid or semisolid media, and 10 per cent gelatin solutions were found adequate for this purpose. The material was suspended in warm 10 per cent aqueous gelatin solution, a small amount of formaldehyde added, and the solution allowed to gelatinize by cooling. Such gels, under the action of formaldehyde hardened to such a degree that within a few days they would not melt at any room temperature.

The substance which we found most satisfactory for suspending in gelatin was discovered by accident when testing the preserving action of hydrazin sulphate and hexamethylenetetramine in the same specimen of urine (Kingsbury and Williams). An amorphous white material closely resembling the protein sulphosalicylate formed when an albuminous urine was precipitated by sulphosalicylic acid was noted in the sediment of this urine. We then determined the best conditions for preparing this substance from pure solutions of hydrazin sulphate and hexamethylenetetramine and found that equal volumes of aqueous 1 per cent solution of the former and 10 per cent solution of the latter gave the most suitable product.

In looking up the literature it was found that Pulvermacher[3] had made a compound in 1893 from formaldehyde and hydrazin hydrate with which this is probably identical. We have abbreviated *formalazin,* Pulvermacher's name for this compound, to *formazin.* Elementary analyses were made for us by S. J. Dobrovolny under Dr. W. H. Hunter's direction at the University of Minnesota. (Table I.)

TABLE I

	FORMAZIN, (HEXAMETHYLENETETRAMINE, + HYDRAZIN SULPHATE) KINGSBURY AND WILLIAMS		FORMAZIN, (HYDRAZIN HYDRATE + FORMALDEHYDE) PULVERMACHER	
	I	II	III	I
Carbon	41.85%	40.28%	38.85%	42.3 %
Hydrogen	7.84%	7.41%	7.08%	7.11 %
Nitrogen	48.76%	46.66%	46.60%	50.06 %
				49.85 %

Formazin is difficult to purify and the samples sent to Dr. Hunter were not as uniformly pure as we could have wished, which accounts for the slight discrepancies in the analytical figures. It seems reasonable to believe that, irrespective of the methods of preparation, the two substances are chemically identical, although physically quite different, for, made by Pulvermacher's directions, formazin is a gelatinous substance resembling aluminum hydroxide cream and cannot be used for our purpose, that is, suspending it in gelatin in such a way as to resemble closely a dispersion of urinary albumin sulphosalicylate. In our method of making formazin the main factor appears to be

the mild acidity, a P_H of about 6.3, which produces a mixture with a constantly low concentration of formaldehyde. Formazin is therefore formed very slowly, and a suitable product is so obtained.

Formazin, as we make it, can be suspended in close imitation of a urinary albumin sulphosalicylate dispersion in the following media: water, glycerin, ethyl alcohol, lactic acid, glacial acetic acid, amyl alcohol, oleic acid and paraffin oil. In this respect it is the most remarkable substance with which we are familiar. The only medium, however, which we have found in which formazin can be suspended and retain its original degree of turbidity unchanged, is a gelatin gel; at least this is the only one that has proved adequate in our hands.

PREPARATION OF GELATIN-FORMAZIN STANDARDS

1. *Preparation of the Gelatin Solution.*

The grade of gelatin which we have found best suited is the Super X brand of the Coignet Chemical Products Company, 17 State Street, New York City. The sheet form is more easily brought into solution than the granulated. Fifty grams of sheet gelatin are placed in a large beaker with 350 c.c. of distilled water. Working time will be saved in bringing this into solution by allowing it to stand over the preceding night in the incubator at 38° C. It is then dissolved by heating in a water-bath at 45° to 50° C. and made up to 500 c.c. with distilled water in a graduated cylinder. The white of one egg is now stirred in and the mixture heated in a boiling water-bath for at least an hour. A clearer product will be obtained if the coagulated egg white is not broken up by stirring. The gelatin solution is filtered hot through a Whatman's No. 4 filter paper, the large clots of albumin also serving as a filter, and allowed to cool. When cool it is put on ice until used. The perfectly clear gelatin gel so prepared must be used within a few days, since no preservative has been found which does not interfere in the subsequent steps of the procedure.

2. *Preparation of the Formazin Suspension.*

Two and one-half grams of hexamethylenetetramine* (urotropin) are dissolved in 25 c.c. of distilled water at room temperature. One-fourth gram of hydrazin sulphate* is likewise dissolved in 25 c.c. of distilled water at room temperature, and the two solutions are mixed in a small flask by pouring the hexamethylene solution into the hydrazin sulphate solution. The flask is then stoppered. In the course of two or three hours the clear, colorless solution becomes turbid and fifteen to eighteen hours later, when the reaction has come to an equilibrium, is ready for use. There will then be a considerable quantity of amorphous white precipitate present which will be uniformly suspended on gently inverting the flask a few times. We have found that the turbidimetric value of this suspension is constant and that it may be used for a period of some months, provided that it is kept stoppered.

*These chemicals and sulphosalicylic acid may be obtained from the Research Laboratory of the Eastman Kodak Co., Rochester, N. Y.

3. *Preparation of the Standards.*

Immediately before using, the 10 per cent gelatin gel is warmed to about 40° C., which liquifies it, and 0.3 c.c. of formalin (40 per cent formaldehyde solution) is added to each 100 c.c. and thoroughly mixed in. Care must be taken not to add an excess of formalin, as this will cause a gradual increase in the turbidity of the standards in the course of time which will render them useless. The 100 mg. standard, equivalent in turbidity to that produced when the corresponding or 0.1 per cent albumin standard is precipitated with three volumes of 3 per cent sulphosalicylic acid, is made by adding 14.5 c.c. of the freshly agitated formazin suspension to 100 c.c. of 10 per cent gelatin solution. This operation is carried out at 40° to 45° C., and care is taken to make the mixing thorough. The stock 100 mg. standard is then diluted with 10 per cent gelatin solution to make the lower standards. (Table II.)

TABLE II

FORMAZIN-GELATIN SUSPENSION, EQUIVALENT TO 100 MG. ALBUMIN PER 100 C.C.	10% GELATIN	STANDARD PRODUCED
25 c.c.	26 c.c.	0.05% or 50 mg.
20 c.c.	30 c.c.	0.04% or 40 mg.
15 c.c.	35 c.c.	0.03% or 30 mg.
10 c.c.	40 c.c.	0.02% or 20 mg.
5 c.c.	45 c.c.	0.01% or 10 mg.

Each standard when made is poured into an appropriate test tube, sealed carefully with a waxed stopper and the top paraffined. In very hot weather it is best to keep the standards in a cool place for a few days, after which the gelatin has hardened to such a degree that it will not melt at any room temperature.

It is essential that the test tubes which contain the permanent standards should have the same internal diameter and wall thickness as those used in the actual precipitation of the urines.

Pyrex test tubes have been found wholly satisfactory, but soft glass tubes may also be used and are easier to obtain in quantity in uniform bore. The internal diameter of the tube should be 12 to 14 mm. A convenient length of tube is 125 mm. It is necessary that all tubes be fairly uniform in bore; that is, should not vary more than 0.5 mm. Pyrex tubes of uniform inside and outside diameters with these diameters etched on the tube to facilitate replacements in case of breakage, with or without the graduations at the 2.5 and 10 c.c. levels may be obtained from R. G. Cargille, 74 Cortlandt Street, New York. Tubes of the softer glass, 12 mm. internal diameter and varying by not more than plus or minus 0.1 mm. have been in use in this laboratory for some time and may be obtained from the Emil Greiner Company, New York, or the Arthur H. Thomas Company, Philadelphia.

The data given above for the preparation of permanent gelatin standards was obtained by making comparisons with standard serum protein solutions precipitated by adding 7.5 c.c. of 3 per cent sulphosalicylic acid to 2.5 c.c. of the protein solution. At the end of ten minutes the turbidity in the protein

standard reaches its maximum. It is necessary to allow this time for consistent results. We have always made use of three serum proteins standards for each gelatin standard. For instance, to make our 50 mg. gelatin standard as accurately as possible, we compare it with the 45, 50 and 55 mg. serum protein standards, selecting that dilution of gelatin-formazin which is closest to the 50 mg. tube. The comparisons are made in the Clark* lamp. This lamp is not absolutely essential but is desirable, since the comparisons are more easily made with it than by daylight. The tubes are lighted nephelometrically, but a strip with a black line at the bottom of the rack enables the observer to make the comparison by transmitted light, or turbidimetrically. When daylight is used it is necessary to hold the tubes in front of a window, and a black background must be provided. This is simply done by attaching such a background to the back of the rack. Photographs of this rack and Clark's lamp are shown. (Fig. 1.) All the values for albumin by the turbidimetric or nephelometric method were obtained with Clark's lamp.

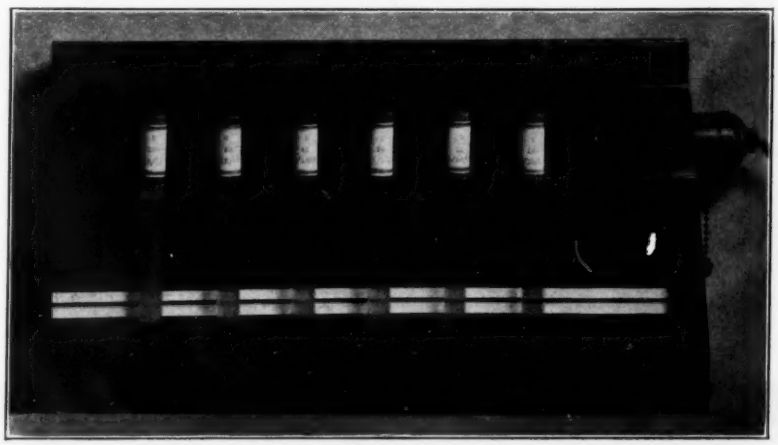

Fig. 1.

THE DETERMINATION OF ALBUMIN

Two and one-half c.c. of urine are pipetted† into a test tube, 3 per cent sulphosalicylic acid added from an aspirator bottle to the 10 c.c. mark, the contents mixed by inverting the tube, but not by shaking, and the tube allowed to stand ten minutes. It is then compared with the formazin-gelatin standards. This is best done by arranging the standards in the order of their values in the test tube rack, leaving one hole between each two standards. It will be found that

*Clark's Lamp may be obtained from Dr. Charles P. Clark, Mutual Benefit Life Insurance Co., Newark, N. J.

†An ordinary Mohr 5 c.c. pipette graduated in tenths of cubic centimeters or a special pipette (Fig. 2) of the shape devised by Folin and modified to meet the needs of this laboratory may be used for this purpose. The special pipette has a capacity of about 7 c.c., is graduated 0 at the top, then 1 c.c. and finally at 3.5 c.c. One c.c. of the urine is drawn off for the sugar determination, 2.5 c.c. or to the 3.5 c.c. mark for the albumin determination, and the rest of the urine, about 3.5 c.c., returned to the original specimen bottle. Without rinsing or practical need to rinse, the next urine is drawn up into the pipette and the measuring process repeated. Although the two samples of urine are measured in the upper portions of the pipette, the actual samples delivered are from the bottom portion or that part freshly rinsed by the same urine in the process of filling the pipette. These pipettes, according to the above specifications, may be obtained from the Emil Greiner Co., New York City.

if the urine tube has been put in such position that it is out of place with respect to the order of standards it will stand out quite plainly, but that if it is between the appropriate standards it will blend with them. In this way the comparisons of known with unknown are easily and quickly made.

The value of the standard most closely resembling the precipitated urine is the value reported. The agreement between technicians analyzing the same specimens is very good, and when different values are reported it is nearly always because the specimen in question fell about half way between two standards and could be reported equally well by either. With large numbers of specimens (this laboratory analyzes from 65,000 to 70,000 a year) this method is sufficiently accurate for the collection of reliable statistical data on the excretion of albumin. Certain other life insurance laboratories including those of the Equitable Life, the Mutual Benefit, the Mutual Life of New York, the

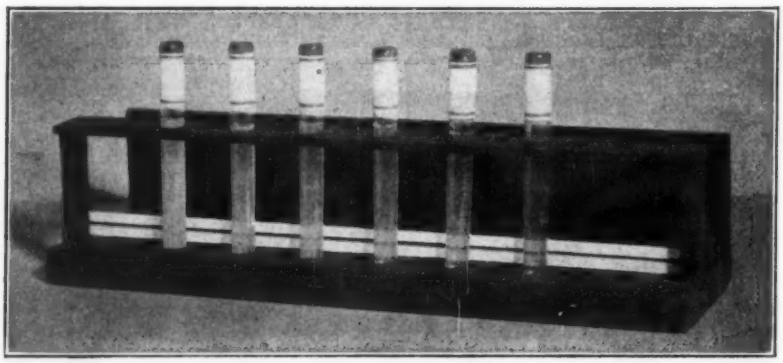

Fig. 2.

Fig. 3.

John Hancock Mutual and several others are using this method as described with permanent formazin-gelatin standards which we prepared.

The serum protein standards used in checking the formazin-gelatin standards are made by allowing whole sheep blood to coagulate, centrifuging the serum free from all cells and determining the total nitrogen by the Kjeldahl method. Multiplying the total nitrogen figure by 6.25 gives the protein (albumin + globulin) value of the serum. From the protein value so obtained a 1 per cent stock solution is made by diluting the serum with the required amount of 15 per cent sodium chloride solution. This solution is then diluted with distilled water to one-tenth its value or to a protein content of 0.1 per cent and thus contains 1.5 per cent sodium chloride. From it are made by proper dilution with 1.5 per cent sodium chloride solutions, the 50, 40, 30, 20 and 10 mg. standards. The original 1 per cent stock solution keeps indefinitely. We have analyzed some that had been kept a year without appreciable change

in its protein content. The lower standard solutions, however, must be fairly fresh, and kept on ice at all times when not actually in use.

On the dates shown in Table III the formazin-gelatin standards were compared with freshly precipitated albumin standards that varied by 5 mg. per 100 c.c. above or below each standard down to the 30 mg. level. Below this we used albumin standards varying only 2 mg. The values shown are those of the nearest albumin standard and were made and agreed upon by at least two of us. It will be noted that on June 5 those standards higher than 30 mg. showed increases that on a percentage basis appear large but which, under the conditions of side by side comparison, were hardly more than just detectable. The standards from 30 mg. down showed practically no change. On November 6, five months later all the standards except the 10 mg. show less turbidity than on June 5. Whether these last values are true minima or whether they merely represent a stage in a very gradual loss in turbidimetric value, which may continue indefinitely, there has not been time to determine, and for this reason we do not claim a greater permanence for them than six months.

TABLE III

PERMANENCE AND OTHER CHARACTERISTICS OF FORMAZIN-GELATIN STANDARDS

STANDARDS MADE												
Jan. 12	Jan. 13	Jan. 14	Jan. 23	Feb. 6	Feb. 11	Feb. 14	Feb. 24	Mar. 12	Mar. 21	May 4	June 5	Nov. 6
1925												
100	110	115	120	97	100	100	110	110	110	110	110	100
50	47	47	57	57	50	50	47	47	55	55	55	45
40			37	40	40	37	40	40	42	42	45	35
30	30	30	30	27	30	30	27	30	30	30	30	25
18*	20	20	18	18	18	16	16	18	18	18	18	15
10	10	10	10	10	10	10	11	10	12	10	12	12

*20 mg. standard was broken. The 18 standard is shown in its place.

Standards of this kind have been exposed to the weather on the roof of the building, withstanding changes in temperature of 70 Fahrenheit degrees, that is from 40° F. to 110° F., and they have also been exposed to many hours of brilliant sunshine for over five months without becoming fluid or showing detectable changes in their turbidimetric or nephelometric values. The only detectable effect has been a very slight bleaching of the color of the gelatin. Exposure of 10 per cent gelatin in a quartz tube to 33 hours of concentrated ultraviolet light by Dr. Percy Hodge of the Stevens Polytechnic Institute, shows a dimming of the lustre of the gelatin on the side directly exposed, apparently due to the precipitation of microscopic particles in this area, possibly caused by a local drying out of the gelatin. In glass tubes, however, our standards are permanent under the adverse conditions to which they have been subjected. It has never been possible to detect aggregated particles in any of them with the unaided eye, nor have any of them which had the prescribed amount of formaldehyde present ever shown bacterial decomposition with its accompanying liquefaction.

Microscopic examinations by indirect light of the formazin-gelatin standards and the corresponding freshly dispersed serum protein standards have

been made. Such dark-field studies show that in number and size of particles both standards appear very similar, in fact it is practically impossible to distinguish one from the other. While comparisons of this kind between actual urine tests and the corresponding formazin-gelatin standards show practically the same close resemblance in the majority of cases there are others in which the particles of the albumin sulphosalicylate in the urine appear to be of smaller dimensions than those of the formazin-gelatin and the freshly dispersed serum protein standards.

It should be noted that the color of the formazin-gelatin standards is similar to that of the urine precipitated with 3 per cent sulphosalicylic acid in the proportion used. This makes the turbidity comparison easier than is the case when precipitated sheep serum standards, which are white, are used against the slightly tinted urine tests.

We have compared the values obtained by the use of formazin-gelatin standards with those obtained gravimetrically by heat coagulation. The method we used for this, while similar to that described by Folin and Denis[1] as a check method, differs from it in that we coagulate the albumin at or near its isoelectric point, P_H 4.7, by adding sufficient sodium acetate-acetic acid buffer solution to produce this result. A few drops of 0.02 per cent methyl red is used as indicator.

In Table IV are shown some determinations by this method of serum standards and albumin free urines to which serum protein in known amount had been added.

TABLE IV

	FOUND BY THE MODIFIED GRAVIMETRIC METHOD MG. PER 100 C.C.
First serum standard, contains 100 mg. protein per 100 c.c. as shown by the total nitrogen determination	100
Second serum standard, also made to contain 100 mg. protein per 100 c.c.	94
Albumin free urine to which serum protein had been added to make it 50 mg. per 100 c.c.	53
Albumin free urine to which serum protein had been added to make it 200 mg. per 100 c.c.	201

In Table V are shown some comparative figures obtained with formazin-gelatin standards and the modified gravimetric check method. The urines were not selected except to contain at least 20 mg. albumin per 100 c.c. The formazin-gelatin standards used were equivalent to the following amounts of protein in milligrams per 100 c.c. of solution: 10, 20, 30, 40, 50 and 100. The values given are those of the nearest standards. The plus and minus signs indicate that the values although closest to the standard given are slightly above or below it.

It will be noted that the sulphosalicylic acid method with formazin-gelatin standards agrees well with the gravimetric method when one remembers the inherent difficulties in any nephelometric method and the difficulty of completely removing, purifying and weighing the very small amounts of albumin actually dealt with in the gravimetric method.

TABLE V

LABORATORY NUMBER	BY FORMAZIN-GELATIN STANDARD, MG. PER 100 C.C.	BY THE MODIFIED GRAVIMETRIC METHOD MG. PER 100 C.C.
8153	50	40
8165	20+	18
8134	100–	93
8175	50	37
8285	50–	44
8228	30–	26
8283	50+	53
8356	30+	30
8386	40–	36
8388	40+	36
8404	30–	32
8411	50–	42
8428	100–	71
8454	50	43
8457	100	101
8475	50–	46
8505	40–	39
8521	40+	45
8546	50+	60
8591	30–	21
8617	40+	32

CONCLUSION

An improved sulphosalicylic acid nephelometric or turbidimetric method with permanent standards is described. This method is of sufficient accuracy for use in the collection of data in the routine examination of urines for the smaller amounts of albumin.

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SIMPLIFIED TECHNIC FOR THE SHAFFER-HARTMANN METHOD FOR BLOOD-SUGAR ANALYSIS*

By E. P. Bugbee, M.D., and A. E. Simond, B.S., Detroit, Mich.

AFTER having done several thousand determinations of glucose in standard solutions and in blood we have adopted some modifications of the Shaffer-Hartmann method.[1] These modifications do not introduce errors which are appreciable, in view of the fact that the original method is subject to errors of 10 per cent or more, depending upon the technic of the analyst.

Formerly we standardized our sodium thiosulphate solution against pure iodine or iodine liberated from potassium iodate by the addition of potassium iodide and sulphuric acid. Later we made use of the modification introduced by Haskins and Holbrook.[2] In this modification the thiosuphate is standardized against the Shaffer-Hartmann copper reagent. It is then adjusted so that the thiosulphate instead of being exactly N/200 is of such strength that 19.50 c.c. are required to decolorize 5 c.c. of the copper reagent. This is a great labor saving modification in two ways: (1) it makes the preparation of the thiosulphate much easier, and (2) it makes possible the use of tables or graphs reading in terms of c.c. of thiosulphate used in titration, rather than difference between the blanks and thiosulphate used.

If pure chemicals, carefully prepared solutions, and uniform technic are used, the method of Haskins and Holbrook gives results which are accurate enough for clinical purposes. We demonstrated this fact by doing analyses of a series of standard glucose solutions. Three or four determinations were done on each standard solution. The percentage of error is shown in Table I.

Table I

Error Made in Analyses of Glucose Solutions by the Method of Haskins and Holbrook

Glucose Solution	Analysis	Error	Glucose Solution	Analysis	Error
%	%		%	%	
Blank	0	0	.225	.225	0
.010	.003	−70%	.250	.245	−2%
.020	.006	−70%	.275	.268	−2.5%
.030	.017	−43%	.300	.292	−2.6%
.050	.044	−12%	.325	.305	−6.1%
.075	.068	− 9%	.350	.340	−2.9%
.100	.093	− 7%	.375	.380	+1.3%
.125	.120	− 4%	.400	.380	−5%
.150	.149	−0.7% and −2%			
	.147				
.175	.168	− 4%			
.200	.197	−1.5% and −0.5%			
	.199				

It will be seen by consulting Table I that the errors are enormous for very dilute glucose solutions. For this reason no dependence should be placed upon figures obtained by this method for glucose solutions or blood sugar more dilute than .04 per cent.

Formerly, when doing blood-sugar analyses in dogs, we drew about 3 c.c.

*From the Research Laboratory of Parke, Davis and Company.
Received for publication, November 28, 1925.

of blood for each determination. This was put into a bottle containing two drops of 10 per cent potassium oxalate. Then exactly 2 c.c. of this oxalated blood was pipetted out and added to the water, sulphuric acid and sodium tungstate, according to the Folin-Wu[3] method for preparing the clear protein-free filtrate. When we worked with rabbits we found difficulty in obtaining enough blood, so we tried to get along with smaller samples. We found we

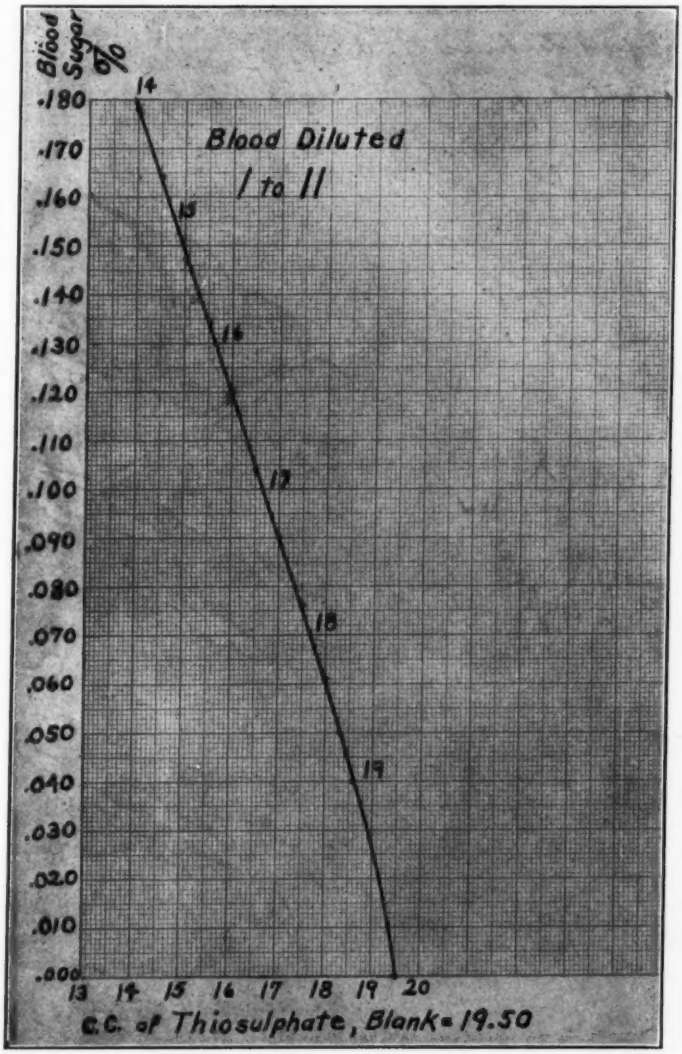

Fig. 1.—Shows per cent of blood sugar in blood diluted 1 to 11 corresponding to c.c. of thiosulphate used in titration.

could draw the blood directly from the rabbit's heart by means of a well oiled 1 c.c. tuberculin syringe. If the heart is pierced near the apex it causes no trouble, but if the base of the heart or the large vessels are pierced, the animal is liable to bleed to death. A tuberculin syringe is quite accurate, and if the same syringe and needle (22 gauge 1¼ inches long) are used for each sample any error is constant.

Haden[4] recommended adding 1 c.c. of blood directly to 8 c.c. of N/12

sulphuric acid and then adding 1 c.c. of 10 per cent sodium tungstate to precipitate the protein. This shortens the process to the use of two solutions instead of three and apparently works just as well.

We found difficulty in obtaining 5 c.c. of filtrate when only 1 c.c. of blood was used. We wished to use as large a volume of the filtrate as possible so as to reduce errors proportionately. We were able to do this by adding another

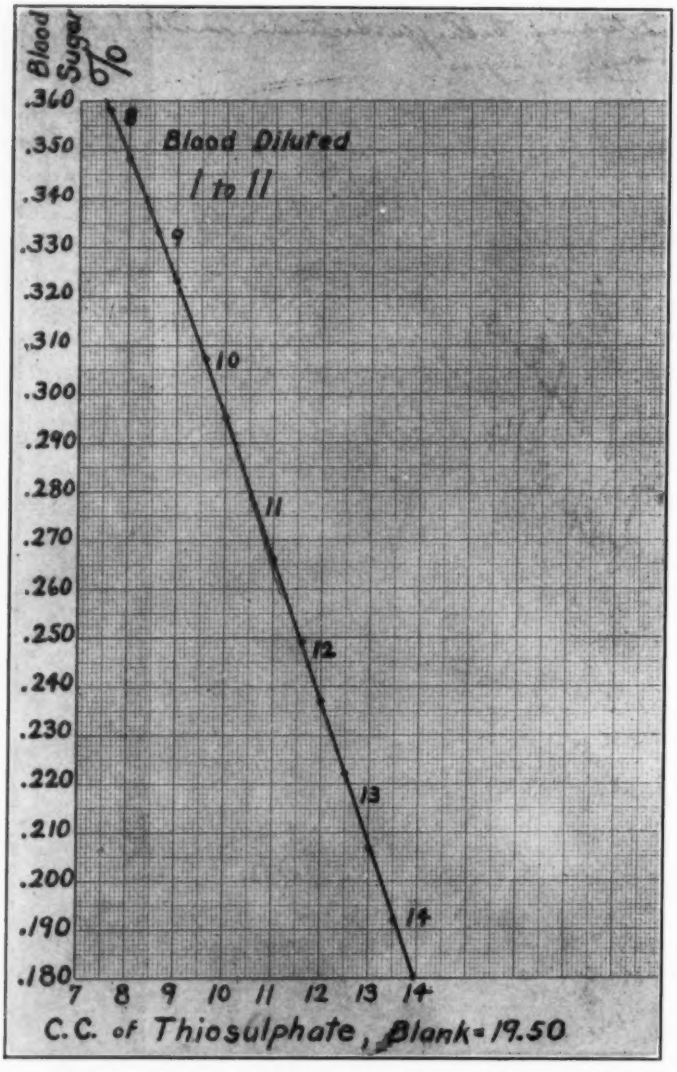

Fig. 2.—Shows per cent of blood sugar in blood diluted 1 to 11 corresponding to c.c. of thiosulphate used in titration.

1 c.c. of water, which made our dilution 1 to 11 instead of 1 to 10. We constructed tables and graphs based on this dilution so that it made the final computation of blood-sugar percentage no more difficult. The extra 1 c.c. of water was combined with the sulphuric acid so that now we use 9 c.c. of 2/27 N H_2SO_4.

We found that it is advisable to do determinations on standard sugar solutions and on blanks at frequent intervals so as to check up on the reagents

as well as on our technic. Sugar solutions may be diluted 1 to 11 instead of 1 to 10 so as to use the same graph as is used for blood sugars.

In constructing our table and graphs we have made use of the figures obtained by Shaffer and Hartmann,[1] because they used purer solutions of glucose than we had at hand. As Haskins and Holbrook[2] also used these figures, our table and graphs may be most conveniently constructed by multiplying the figures given by Haskins and Holbrook by 1.1. Our graphs, Fig. 1 and Fig. 2, are made by drawing smooth curves through points representing values of blood sugar corresponding to the number of cubic centimeters of the sodium thiosulphate used in titration. For directions regarding the method of doing the analysis and for preparation of the solutions reference should be made to the article by Haskins and Holbrook.[2]

SUMMARY

The following modifications of the original Shaffer-Hartmann method for blood sugar have proved to be advantageous.

1. Use of thiosulphate standardized against the copper reagent so that 19.50 c.c. of thiosulphate is required to decolorize 5 c.c. of the copper reagent, as recommended by Haskins and Holbrook.

2. Drawing blood directly into a well oiled 1 c.c. tuberculin syringe so as to do away with the necessity of using anticoagulants and pipettes.

3. Delivery of the blood from the syringe directly into dilute sulphuric acid, as recommended by Haden.

4. Diluting the blood to 1 to 11 instead of 1 to 10 so as to get 5 c.c. of filtrate.

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[2]Haskins, H. D., and Holbrook, W. P.: Jour. Lab. and Clin. Med., 1923, viii, 747.
[3]Folin, O., and Wu, H.: Jour. Biol. Chem., 1919, xxxviii, 81.
[4]Haden, R. L.: Jour. Biol. Chem., 1923, lvi, 469.

A METHOD OF SKIN SCARIFICATION

By Evans B. Wood, M.D., Atlanta, Ga.

AN ORDINARY dental burr will be found to be a very satisfactory instrument for scarification in making skin tests for sensitization, von Pirquet, smallpox vaccination, and like procedures. Scarification is accomplished by grasping the dental burr between the thumb and index finger, resting the cutting end of the instrument on the skin to be scarified, and giving the instrument a twist.

The instrument is easily sterilized either by flaming or by boiling. It is easily procured and very inexpensive. Various sizes may be had from any dental supply house.

This method of scarification has the advantage of producing a uniform area of scarification; the process is almost painless; children are not frightened, and there is little danger of producing any accidental injury. The time required for vaccination is reduced to a minimum when large numbers have to be handled.

HYDROGEN-ION TITRATION OF MEDIA AND PREPARATION OF COLOR STANDARDS*

By B. W. Culyer, Ottawa, Canada

AFTER experimenting with various methods[1, 2, 3, 4] with more or less success, the following technic for the determination of hydrogen-ion concentration as applied to bacteriologic media, has, for the past few years, given every satisfaction in this laboratory. Upon the completion, a year or two ago, of some work on the buffer indices of bacteriologic media, following the technic of Brown,[5] it was thought that his method of titration might easily be adopted as a routine procedure for the titration of culture media. After the initial difficulties experienced in the general preparation of buffer solutions and colorimetric standards have been overcome, this method of Brown, so clear cut and simple, will, I am sure, appeal to most laboratory workers as worthy of general adoption.

METHOD OF TITRATION OF MEDIA

I. *Equipment.*—

a. A selected set of colorimetric hydrogen-ion standards is taken; the following series will cover the ranges necessary for all ordinary culture media:

Brom Cresol Green	4.2–6.0
Brom Thymol Blue	6.0–7.6
Phenol Red	6.8–8.4
Cresol Red	7.2–8.8

b. Tubes of nonsoluble glass, graduated to 10 c.c. and of the same size as those containing the standards. Tubes specially designed for this purpose, as well as tubes for colorimetric standards, already drawn out for sealing, may now be obtained from the Arthur H. Thomas Co., of Philadelphia.

c. A comparator block.

d. Bottles containing the indicators.

e. A dropping pipette with rubber teat and of the same bore as that used in the preparation of the standards.

f. Normal and twentieth normal solutions of NaOH and HCl.

g. Distilled water.

h. Very accurate pipettes of 1 c.c. capacity, and of 0.1 c.c. capacity graduated in hundredths.

II. *Titration.*—

a. Into each of 2 tubes place 1 c.c. of the medium to be titrated and fill up to the 10 c.c. mark with distilled water. It is to be noted that, if titrating agar, the distilled water should previously be made quite hot to allow for a true distribution of the medium in the fluid. Before a reading is made after the contents are well mixed, the tube should again be cooled.

*From the Laboratory of Hygiene, Department of Health, Ottawa, Canada.
Received for publication, February 6, 1926.

b. To one of the tubes (Tube 1) add the same number of drops of indicator as was used in the preparation of the standards chosen for the titration. The other tube containing the medium (Tube 2) is used as a correction screen.

c. Place in the comparator block the standard of the P_H value required; in front of this, place the correction screen (Tube 2); the unknown (Tube 1) is placed alongside and is backed by a tube containing distilled water. A reading will now determine whether the addition of acid or alkali is necessary to obtain a color match. If the unknown does not now match with the standard, either N/20 NaOH or N/20 HCl, as the case may be, is cautiously run into the tube by means of a 0.1 c.c. pipette graduated in hundredths, mixing well after the addition of each drop, until a perfect color match is made. The amount of N/20 used, if multiplied by 5, will give the percentage of a normal solution required to bring the bulk of the medium to the desired P_H value. After addition of the requisite amount of normal acid or alkali to the medium, the test is repeated until finally the sample shows no need of further adjustment.

THE PREPARATION OF COLORIMETRIC HYDROGEN-ION STANDARDS

The buffer solutions and the stock indicators are prepared according to the method and technic of Clark.[6] It is possible to keep the buffer solutions in good condition over a long period by storing in pyrex glass bottles which are pasteurized and sealed with "Viscose" bottle caps. From these are made up the sets of colorimetric hydrogen-ion standards. The procedure is as follows:

A sufficient number of the kind of tubes already specified, enough to accommodate a series within the range of the indicator selected, is placed in a rack. These tubes are to be cleaned carefully, sterilized by heat, and into each is measured accurately, by means of a certified pipette, 10 c.c. of each buffer combination within the range required. A clean, dry pipette should be used for each operation. The next step is the addition of the indicator. This is added by means of an ordinary dropping pipette with rubber teat; several of these, each of exactly the same bore, should be at hand, so that, in case of an accident in the middle of the operations, the pipette may be replaced without variation in the size of the drops. The same number of drops are added to each tube, starting at 5 and working up drop by drop to each, until the point is reached where the desired degree in coloration is attained. The number of drops used should be recorded and later placed on the labels of the standards. With some indicators 7 drops have been found sufficient; with others, as many as 14 drops have been used. The tubes are then placed in a block for comparison; each tube with the next higher and the next lower P_H value. For this purpose a comparator block having three slots for tubes has been found useful. Starting at the lower end of a series, after checking the first three tubes, the left-hand one is removed and the remaining two moved over to the left. Then the fourth tube is placed in the vacant slot and so on. Any error, either in the preparation of the buffer solutions, or in general technic will soon become apparent. If the grading of the series is found to

be correct, the tubes are then sealed off in a flame and heated in a water-bath for thirty to forty minutes at 75° to 80° C., as a precaution against the development of moulds, which, if they do not alter the P_H and thus the color of the standards, render it difficult to make clear-cut readings. After being labelled, the colorimetric standards are stored in a cabinet specially constructed with racks and made to exclude light.

ADVANTAGES OF THIS METHOD

1. By this method, the titration of any highly colored medium is rendered relatively easy and accurate, owing to the high dilution of the medium.

2. The method allows of titrating agar in a liquid, or at least in a highly dispersed state.

3. It is possible, in the majority of instances, by using one tube only, both to establish the primary reaction and to adjust it to the desired end-point.

4. Any reading when multiplied by 5 is in direct terms of N/1 per cent.

5. By using tubes of nonsoluble, clear, white glass and by pasteurizing after sealing, the standard tubes may be kept in perfect condition, practically indefinitely.

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[6]Clark, W. M.: The Determination of Hydrogen-ions, ed. 2, Baltimore, 1922, Williams & Wilkins Co.

ALARM APPARATUS FOR ARNOLD STERILIZER*

By John W. Churchman, M.D., and Louis Siegel, New York City

IN EVERY laboratory in which Arnold sterilizers are used, annoyance and often expense result from the fact that the apparatus, through inadvertence, is allowed to boil dry. To prevent this accident the following simple device has been installed in our laboratory. Since it has proved its value by successful operation for some time, it appears worth while to describe it.

The apparatus requires few parts and these can be purchased in any electrical supply store. It is inexpensive, costing approximately as follows:

1 Piece asbestos board	$ .10
2 Dry batteries	.70
1 Electrical bell	.45
1 Rubber ball	.10
3 Feet insulated bell wire	.03
2 Feet cotton thread	.01
	$1.39

*From the Laboratory of Experimental Therapeutics, Cornell University Medical School. Received for publication, February 7, 1926.

The apparatus consists essentially of an open electrical circuit, connected to a bell, and a gravity float on the surface of the water in the Arnold. In Fig. 1 the alarm apparatus is shown in solid lines, the Arnold sterilizer in broken lines. The alarm apparatus is placed on top of the Arnold. The batteries should stand on a piece of asbestos to protect them from the heat.

The two batteries (*A*) and (*B*) are connected in series so as to get 3 volts, and one terminal is connected to one binding post on the electrical bell

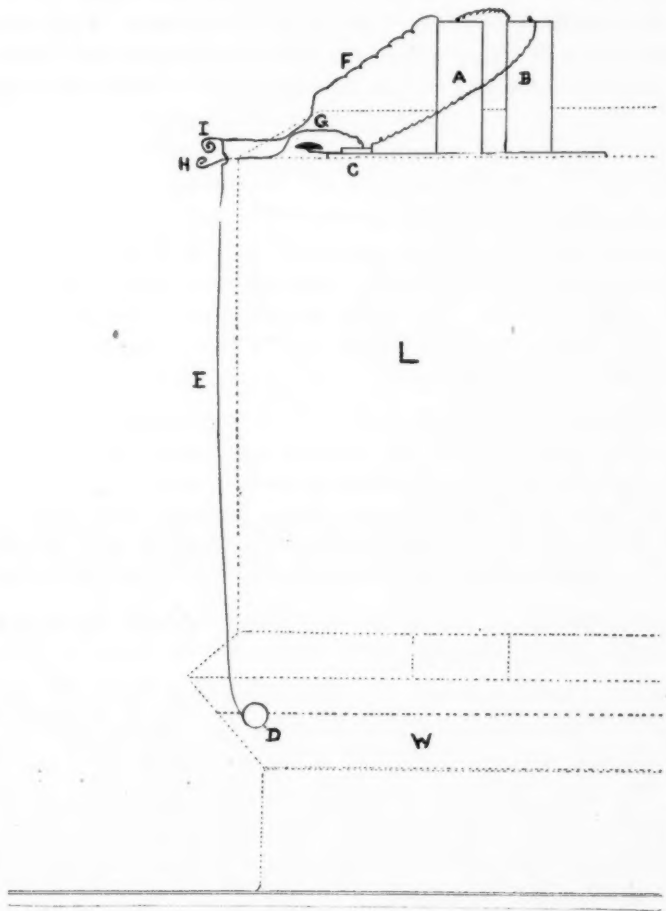

Fig. 1.—*L*, Arnold sterilizer; *W*, water in Arnold; *D*, rubber ball float; *E*, thread (a curve has been drawn in thread *E* near wire *G* to indicate that the thread must be so arranged as not to touch the wire; *A*, *B*, batteries; *C*, electric bell; *F*, wire from battery *A*; *G*, wire from bell; *I*, coiled contact surface, wire *F*; *H*, coiled contact surface, wire *G*. The batteries have been indicated in the upright position for the sake of clarity; it is actually more convenient to lay them on their sides.

(*C*). To the other terminal of the battery a wire (*F*) is attached. This wire rests on the top of the Arnold and runs for a short distance beyond its edge. To it is attached a thread (*E*) leading to a rubber ball (*D*) which floats on the surface of the water in the Arnold water cell. From the distal end of the wire (*F*) the insulation is removed for a length of about two inches and the uninsulated portion is coiled so as to make a large surface for contact (*I*). From the other terminal of the electrical bell a wire (*G*) is run along the top

of the Arnold and a few inches beyond the edge of the top. From the end of this wire the insulation is removed for a length of about two inches and the uninsulated portion is coiled so as to make a large surface for contact (*H*). The contact points (*H*) and (*I*) are so arranged by bending the wires that when the Arnold is full of water there is a space of about one inch between them. As the water in the Arnold (*W*) boils away and its level falls, the rubber float (*D*) falls with it. The thread (*E*) attached to the wire (*F*) tightens; contact point (*I*) is lowered, and, when the water level gets below a certain point, contact between (*H*) and (*I*) occurs. This contact closes the circuit and the bell rings. It continues to ring until the water is replenished. The addition of water raises the float (*D*). Wire (*F*), released from

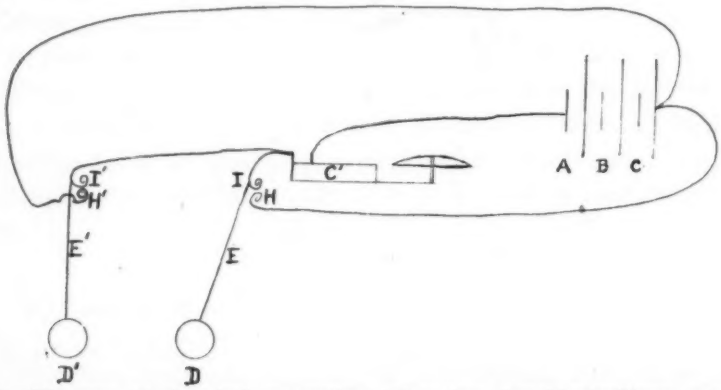

Fig. 2.—Diagram of electrical connections when two Arnold sterilizers are to be controlled by one alarm. *A, B, C*, batteries (three cells should be used for two sterilizers); *D, D'*, rubber ball floats of the two sterilizers; *E, E'*, connecting threads for the two sterilizers; *H, H', I, I'*, contact points for the two sterilizers; *C'*, bell (one bell for both sterilizers).

the pull of the thread, is raised by the spring of the wire; the contact is broken, and the bell ceases to ring.

The apparatus can be set for any desired level of water by simply arranging the length of the thread (*E*) connecting the rubber ball (*D*) with wire (*F*), so that contact between (*H*) and (*I*) cannot occur until the desired level is reached. We have found it useful to regulate the alarm so that it rings when the Arnold is half full of water. This allows about three hours leeway before the sterilizer can boil dry, during which time the bell will ring constantly until the Arnold is refilled.

One alarm may be used for two Arnold sterilizers simply by connecting a second rubber float to the apparatus as shown in Fig. 2.

A NOTE ON THE MASS CULTIVATION OF GONOCOCCI FOR VACCINE*

By Joseph W. Smith, Jr., M.D., and Bert Cross, Washington, D. C.

THE cultivation of gonococci on a large scale for making stock gonococcus vaccine has been somewhat difficult. It has necessitated the use of a large amount of medium and has been a rather laborious process. Attempts to grow gonococci on Kolle flasks, using Vedder's starch agar alone, proved unsatisfactory. We ascribed our lack of success by this method to the fact that the large, flat, open surface of the medium allowed of too free access of oxygen and too rapid drying. Therefore, to obtain conditions of moisture and lowered oxygen tension, the following method for cultivating these organisms in large numbers was devised. It can readily be seen that there is a great saving both in medium and in labor.

The media used are: (1) 5 per cent starch in 3 per cent veal agar, P_H 7.8; (2) veal infusion broth, P_H 7.8; (3) Vedder's starch agar slants for seed cultures. Two hundred mils of 5 per cent starch agar in a one liter Erlenmeyer flask is sterilized and allowed to solidify, and, with aseptic precautions, three hundred mils of veal infusion broth is added to the agar flask. It is well to allow the agar to stand twenty-four hours before adding the broth. The flask is now ready for inoculation.

To inoculate, suspend the growth on a seed culture slant in a few mils of broth, transfer to the flask and incubate at 37° C. for one week. At the end of the week a heavy growth of the organism will be found suspended in the broth near the surface of the agar. To remove the bacteria agitate the flask with a whirling motion, taking care not to shake vigorously enough to break the agar. With aseptic technic, transfer the broth suspension to large centrifuge tubes and centrifugalize until the broth is clear. Pour off the supernatant fluid and suspend the bacteria in salt solution. Heat at 56° C. for thirty minutes and preserve with 0.25 per cent tricresol or 0.5 per cent phenol. Standardize by the opacity method or with the aid of the nephelometer. One flask will produce from 2 to 4 liters of vaccine containing approximately 1000 million organisms per mil.

*From the Department of Clinical Pathology and Preventive Medicine, Army Medical School, Washington, D. C.

Received for publication, February 5, 1926.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Wright, A. E.: New Methods, Devices, and Apparatus for the Routine and Research Laboratory. The Lancet, London, Jan. 2, 1926, iv.

The following are described:

Cover-glass Cell: For the display or enumeration of blood cells, bacteria, or bacterial colonies.

A small pellet of vaseline is placed on a slide and melted by gentle heat. While the vaseline is still liquid, a cover-glass held in a pair of forceps is passed through the vaseline edgewise. When the cover-glass thus edged with vaseline is imposed upon a drop of fluid the escape of the fluid is prevented and the device is termed a simple cover-glass cell.

Hinged and Walled Cover-glass Cell:

A glass slide is cleansed and passed through the flame to sterilize it and a cover-glass similarly prepared. With a soft paint brush the cover-glass is painted around the edges with melted vaseline, one edge being untouched to facilitate lifting.

The vaseline should overlap the edges a millimeter or two. The vaseline upon the farthest edge is made slightly thicker to form the hinge.

The cell is formed by placing the cover-glass with a vaselined surface downward on the cover-glass.

When it is desired to fill the cell, the cover-glass is raised by the edge without vaseline and will stand upright upon the vaseline hinge mentioned above. When the cell is filled the cover-glass is lowered and sealed by painting additional vaseline around the edges.

Such hinged cells may be used as bacterial culture chambers, for differential counting of living and dead organisms in a culture or similar observations.

When using such a chamber for bacterial counts the uppermost and nethermost layers of the fluid may be indicated by the following simple device.

A piece of fine emery paper (00) is drawn horizontally along the slide with firm pressure, thus marking longitudinal striae on the slide.

By placing a cover-glass upon the emery paper, pressing down with the finger, and moving the glass about one inch, a similar set of striae may be produced. The glass and slide may be so adjusted that small squares may be formed which facilitate the count and indicate the two delimiting planes.

Cram, E. B.: The Egg-producing Capacity of Ascaris Lumbricoides. Jour. Agricult. Res., May 15, 1925, xxx, No. 10, p. 977.

By means of a laborious and painstaking study it is computed that the total number of eggs, fully developed and in process of development, in the female Ascaris lumbricoides is from 26,000,000 to 27,000,000.

Hull, T. G., and Nauss, R. W.: Laboratory Differentiation of Smallpox and Chickenpox. Amer. Jour. Pub. Health, February, 1926, ci.

As a result of rather extensive observations the following conclusions are formulated:

1. Rabbits immunized with vaccine virus will show, upon intracutaneous injection of vaccine virus or material from smallpox lesions, typical allergic reactions within 48 hours.
2. Serum from chickenpox lesions rarely produces allergic reactions in immune rabbits.
3. Chickenpox material does not sensitize rabbits to further injections.
4. Scabs from chickenpox cases usually cause an inflammation on smallpox immune rabbits.

5. Factors producing inconsistent results are individual variations in the animal and variations in different strains of material.

6. The amount of virus injected directly influences the degree of the reaction.

7. Blood serum from an individual highly immunized to vaccine virus will produce an allergic reaction in immune rabbits.

8. Nonpotent vaccine virus will produce an allergic reaction in immune rabbits.

9. Rabbits lose their immunity in 9 to 14 months.

10. Guinea pigs and rats are not suitable for this test.

DeJong, S. I., and Handuray, P.: Concerning a Case of Typhoid Fever: Late Examination of Haemocultures in Nonbiliary Medium: A New Interpretation of the Electivity of the Biliary Media. Bull. et Mem. Soc. Med. d. Hôsp. d. Paris, xli, 1561.

Absence of growth in bouillon after four days is usually interpreted as a negative result. When growth occurs in a bile medium inoculated at the same time, the broth remaining sterile, it has been assumed that the lack of growth was due to the bactericidal power of the inoculated broth.

DeJong and Handuray report a case in which a bile culture made on the sixth day gave a growth of typhoid bacilli, a culture made at the same time in peptone-water remaining negative.

On the seventeenth day, however, the peptone-water contained motile bacilli, morphologically typhoid bacilli which failed to grow on transplanting.

The filtrate of this culture destroyed fresh cultures of typhoid bacilli but failed to destroy typhoid organisms grown in bile.

The authors extol the advantages of bile for blood culture in typhoid and advance the suggestion that bile may be able to prevent the destructive action of bacteriophage.

Herrold, R. D., and Saelhof, C. C.: Skin Reactions with Filtrate of Koch Strain of Bacillus Tuberculosis. Jour. Am. Med. Assn., March 13, 1926, lxxxvi, 747.

The Koch strain is an avirulent culture giving a profuse growth overnight and originally isolated in 1888.

Studies were conducted to determine if the intracutaneous injection of broth filtrates would give a skin reaction.

Skin reactions were obtained in normal adults in dilutions up to 1:50.

The results are tabulated below:

TYPE OF PATIENT	NO.	POS.	DOUBTFUL	NEG.
Advanced tuberculosis	35	0	0	35
Mod. advanced tuberculosis	31	0	0	31
Incipient tuberculosis	8	0	0	8
Apparently normal adults	61	47	8	6
Apparently normal children	14	2	3	9

It is tentatively suggested that the presence of a reaction in the majority of apparently normal adults may indicate that a substance is produced by the growth of this strain in broth which acts in a different way from tuberculin, and that the reaction may mean that, in the presence of an active focus, there may be sufficient antisubstance produced to neutralize the toxic substance.

Smith, J. W., Jr.: Standardization of Typhoid Vaccine. Jour. Infect. Dis., November, 1925, xxxvii, No. 5, p. 385.

Inasmuch as the dosage of bacterial vaccines is customarily expressed in terms of numbers of bacilli per centimeter, the actual enumeration of bacteria becomes the only really practical basic method despite the fact that more accurate methods of standardization are possible and have been proposed.

Smith, therefore, investigated the accuracy of methods of vaccine standardization by bacterial counts.

In the usual Wright method there is a basic error due to the loss of red blood cells accounted for, in large degree, by sedimentation and adherence of the cells to the walls of the counting apparatus and, in lesser degree, to a loss of red cells by hemolysis.

A method of "wet Wright counts" was, therefore, devised as follows:

1. A bacterial suspension is prepared exactly as for the Helber count.

2. Exactly equal volumes of this suspension and the secondary dilution of blood cells are mixed.

3. A drop of this mixture is placed in the Helber chamber and examined.

4. The red cells and bacteria are counted in 40 to 100 small squares.

5. Calculations are made as by the usual Wright method.

In this preparation are obtained a red cell count and a bacterial count.

Even with the "wet method" the same sources of error are encountered though to a lesser degree.

Because of this loss of red cells the ratio of cells to bacteria is not 5:x but 2.5:x or 3:x. Consequently, when the ratio 5:x is employed the bacterial count is overrated and the final suspension is numerically only about one-half as great as it appears to be.

Smith contends, therefore, that the counting chamber (Helber) method is the method of choice and that the expression of dosage should be adjusted in conformity.

Peterson, R. A.: A Simple Blood Coagulometer. China Med. Jour., November, 1925.

The apparatus described consists of a wide-mouthed bottle of 70 to 100 c.c. capacity and about 8 to 12 inches high.

A rubber cork with three perforations carries, in the middle perforation, a clinical thermometer, on either side of which are thrust two inoculating needles each carrying a platinum wire in which four loops have been made, one above the other.

The bottle is filled with water at 100° F. to such a level that the loops clear the water when the stopper is inserted. The loops are filled with blood and one loop at a time thrust into the water until one of the loops remains occluded by fibrin.

The normal coagulation time by this instrument is 4½ minutes.

St. George, A. V., and Brown, A. L.: The Value of the Icterus Index in Differentiating Anemia. Arch. Int. Med., December, 1925, xxxvi, 847.

The authors have modified the Meylengracht method by using his potassium dichromate solution (potassium dichromate 0.05 gm., water 500 c.c., and conc. sulphuric acid 2 drops) as representing 1 and preparing a series of permanent standards of increasing intensity. The necessity for diluting the specimen is thus done away with and the comparison simplified.

From a study of this method in 120 cases they formulate the following conclusions:

1. The normal range of the icterus index falls between 3.5 to 4.5, the average being 4.41 in their series of 120 cases.

2. Rationally interpreted, the icterus index is a distinct aid in the differentiation of anemias, uncomplicated cases of hemolytic anemia having a high range, and uncomplicated secondary anemia having a low range.

3. The severe anemia of carcinoma may be differentiated by its consistently high index (8 to 12.5) from the uncomplicated pernicious anemia which it may simulate.

4. The most common factors increasing the icterus index above that usual for the primary disease are, coexisting pneumonia, cardiac insufficiency and chronic sepsis, eespecially in the biliary system.

The most common factor decreasing the index below the usual level for the primary disease, is hemorrhage, especially a slow, constant bleeding.

5. It is suggested that the icterus index may be used to follow the course of pernicious anemia and to determine, before the blood count falls, when it is advisable to give a transfusion as well as when spontaneous improvement is likely.

Kowarski, A.: A Method for the Determination of Lactose in the Blood. Ztschr. f. klin. Med., Dec. 22, 1925, cii, 382.

The method is based upon the difference between the reduction power of lactose and glucose.

The reducing power of lactose is changed after inversion by boiling with acid; that of glucose remains unaffected. Lactose may be determined, therefore, on the basis of the difference between the reduction power before and after boiling with acid.

Method.—Into a long-necked, flask-shaped, measuring vessel with a capacity of 50 c.c. pour 20 c.c. n/100 acetic acid, 20 c.c. distilled water, and 2.5 c.c. of the serum from the blood which is to be examined. The mixture is placed in boiling water for 1 to 2 minutes, then 3 to 6 drops of n/10 sodium hydroxide added, and the whole again placed in boiling water for five minutes. The albumin coagulates in large clots, and the fluid becomes quite transparent.* The mixture is then allowed to cool, and about 0.5 gm. aluminum silicate added. The flask is filled to its capacity mark, the mixture shaken strongly, and then filtered. The filtrate must be water clear and absolutely free of albumin (control with sulphosalicyclic acid). Then place 20 c.c. of the filtrate corresponding to 1 c.c. of the serum, into each of two large reagent glasses of 50 c.c. capacity. To the first add 1 c.c. 20 per cent sulphuric acid, place the reagent glass in a boiling water-bath, and heat for 1 hour. Then to the contents of the second glass add also 1 c.c. 20 per cent sulphuric acid. To each add 0.4 gm. sodium potassium tartrate, 2.0 c.c. 30 per cent sodium hydroxide, and 2.0 c.c. of a copper sulphate solution which contains sugar (0.1 gm. chemically pure dextrose, 8.0 gm. copper sulphate, and distilled water to 200 c.c.). The salt is then dissolved by stirring, and the reagent glasses heated in the water-bath for five minutes. After the glasses have been cooled for one minute in running water the red oxide of copper which separates out is collected on an asbestos filter and washed. The apparatus required for this is an Erlenmeyer flask which is closed by a two-hole rubber stopper. In one of the holes is an Allihn funnel holding the asbestos filter; and through the other hole the flask is connected with a water pump by a bent glass tube. The stopper with the funnel should also fit an Erlenmeyer flask with a 30 c.c. capacity. The asbestos filter should be made of well-beaten, white, asbestos wool, which is kept suspended in 5 per cent nitric acid. The wool is pressed out with a thick glass rod. The filter is used in such a way that the red oxide of copper is deposited only upon the upper surface, and does not press into the lower layers.

The contents of the cooled reagent glass are poured into the Allihn funnel, and an equal quantity of distilled water added. The flask is then connected with the water pump, and the blue fluid sucked through the filter. As soon as the fluid has passed the asbestos filter the water from the reagent glass is immediately poured into the funnel. (Air suction must be prevented, since this will occasion a further oxidation of the red copper oxide.) Again pour 10 c.c. of water into the reagent glass, and then pour this also into the funnel. Meanwhile, have, in a graduated cylinder, 10 c.c. of an iron sulphate solution (10 gm. ferric sulphate, 40 gm. sulphuric acid, and distilled water to 200 c.c.), and pour 5 c.c. of this also into the reagent glass after the water has been removed. As soon as the washing with water is finished the connection of the flask with the pump is broken, the stopper with the funnel placed in the small Erlenmeyer flask, the fluid from the reagent glass poured into the funnel, and the other 5 c.c. from the graduated cylinder added. The flask is then again connected with the pump, and all the iron sulphate solution passed through the filter. The red copper oxide is completely dissolved by this, and changed into an equivalent quantity of red oxide of iron. The connection with the pump is again broken, and the stopper replaced in the large flask. The filter is now washed with distilled water to remove all the traces of iron sulphate solution from the asbestos. The contents of the flask are titrated with a freshly made solution of potassium permanganate. (Permanent solution contains 1.0 gm. permanganate in 200 c.c. distilled water; titer used is a tenfold dilution of this—5 c.c. to 50 c.c.) The titration is continued until there is a permanent rose color. The burettes used here

*The addition of the sodium hydroxide must be done a drop at a time, because the reaction of the fluid must not become alkaline.

should be graduated in 0.05 c.c. so that quantities as small as 0.025 c.c. can be measured. After the quantity of the potassium permanganate solution used has been noted, the same procedure is repeated with the fluid from the second reagent glass. If the same quantity of permanganate solution is used in both cases there is no lactose present in the blood. If more is used for the tube in which the contents have been boiled with the sulphuric acid, the quantity of lactose per c.c. of blood is found by multiplying the difference by 1.66 mg.

Example: 4.80 c.c. permanganate solution used for the first tube; 5.35 c.c. used for the second tube. The difference is thus 0.55. Multiplied by 1.66 mg. this gives 0.88 mg. lactose per c.c. of blood. That is, 88 mg./100 c.c., or 0.088 per cent.

The amount of dextrose in the blood examined is then determined as follows:

"Multiply the reduction found—in our case 0.55 c.c.—by 10/3. (0.55 x 10/3 equals 1.83). This figure gives the quantity of permanganate which corresponds to the lactose. If this figure is subtracted from the figure which corresponds to the total reduction—in our case this is 5.35—we get the quantity of permanganate used to reduce the dextrose in the blood and in the reagent. (5.35 – 1.83 equals 3.52.) The 2 c.c. of copper sulphate solution in the reagent contains 1 mg. of dextrose. This latter can be determined from time to time in a series of experiments. It remains extraordinarily constant, showing only very small variations of quantities of 2.0 c.c. After the subtraction of this figure, and the multiplication of the remainder by 0.5, we find the quantity of dextrose in mg. per c.c. of blood. In our example the value was 3.52 – 2.0 equals 1.52; 1.52 x 0.5 equals 0.76 mg./c.c. That is, 76 mg./100 c.c., or 0.076 per cent."

The figures computed for the dextrose content are valuable only when a glycolysis has been prevented. Thus, for a simultaneous determination of the dextrose and lactose in the blood, it is absolutely necessary to make the determination immediately after the blood is removed from the patient.

Kendall, A. I.: Intestinal Intolerance for Carbohydrate Associated with Overgrowth of the Gas Bacillus. Jour. Am. Med. Assn., March 13, 1926, lxxxvi, 737.

Kendall describes a syndrome, quantitative rather than qualitative, insidious in onset, and generally associated with some unusual dietary event, though really a culmination rather than a commencement.

The typical symptoms are: constipation, fatigue, meteorism, precordial or hypochondrial pain, sometimes only elicited on deep pressure, hypotension, and intolerance for certain foods.

The etiology is obscure, but gas bacilli, and, less commonly, members of the mucosus group of bacilli of the starch fermenting type are found in unusual numbers which may result in a decrease in the normal number of lactic acid bacilli in the small intestine.

Gilbert, R., and Humphreys, E. M.: The Use of Potassium Tellurite in Differential Media. Jour. Bact., February, 1926, xi, No. 2, p. 141.

A concentration of 1:17,000 of potassium tellurite was found to give excellent results in the isolation of diphtheria bacilli from throat cultures and to have no effect on the virulence of the organisms.

The growth of Gram-positive cocci, certain of the higher bacteria, and yeasts and molds seemed only slightly inhibited, while many of the motile bacilli, including a number of anerobes, were markedly inhibited.

The formula follows:

Beef infusion agar 1.5 per cent	1 liter
Horse serum (sterile)	5 per cent or 50 c.c. per liter
Glucose C. P., 20 per cent solution (sterile)	1 per cent or 10 c.c. per liter
Potassium tellurite C. P., 1 per cent solution (0.58 per cent on test)	1 per cent or 10 c.c. per liter 1:34,000 Te_4 (1-17,000 K_2TeO_3)

Procedure.—Melt the agar and cool to 50° C. Add the serum glucose and tellurite solutions, all previously warmed to 50° C. Mix well and insert a sterile siphon. Dispense in Petri dishes. Incubate the plates and examine for contaminations.

The solution of potassium tellurite is prepared as follows:

Potassium tellurite C. P.	0.25 gm.
Distilled water	25.0 c.c.

Procedure.—Weigh 250 mg. K_2TeO_3 on a chemical balance. Grind to a very fine powder in a small mortar and add about 10 c.c. of the water. Mix well, allow the undissolved portion to settle and pour off the supernatant fluid. Add more of the water to the residue, grind again and combine the two portions. Rinse the mortar with the remainder of the water.

The solution is then passed through a paper filter and finally through the Mandler to sterilize it.

Note.—It is difficult to get this compound into solution and even after thorough grinding in the mortar a small amount of fine suspension is visible. Heating gently to about 55° C. aids the action somewhat.

Elwyn, R., and McMaster, P. D.: Studies on Urobilin Physiology and Pathology. I. The Quantitative Determination of Urobilin. Jour. Exper. Med., April, 1925, xli, No. 4, 503.

The method described is one of comparative fluorescence.

Standard fluorescent solution.

Alcohol 60 per cent	2,000 c.c.
Zinc acetate	50 gm.
Concentrated HCl	2 c.c.

Filter till clear.

Standard fluorescent solution.

Acriflavine	1 mg.
Water	30,000 c.c.

This can be made by appropriate dilution of a 1:1000 solution which is kept in a cool, dark place. The standard solution is stable for one week if kept in the dark.

Method.—The urobilin, recovered from the fluids or substances containing it as described below, is dissolved in the alcoholic zinc acetate solution to develop fluorescence and compared in a comparator with the standard fluorescent solution.

To 15 c.c. portions of the standard solution graduated amounts, as 0.5, 0.55, and 0.6 c.c. of the fluorescent urobilin solution are added, the tubes gently shaken and compared with tubes containing 15 c.c. of standard fluorescent solution.

The nearest color match is noted and the dilution value of the solution under test calculated.

If the unknown diluted is too weakly colored, 0.1 c.c. amounts of urobilin filtrate are added until a match occurs. If the color of the unknown is too intense, a preliminary dilution of the zinc acetate solution is made and this dilution factor multiplied with the final value.

Calculation of Dilution Value.

The dilution value of the fluorescent zinc acetate filtrate must always be multiplied by a factor dependent upon the number of dilutions involved. Thus with bile, 8 is the characteristic factor since 20 c.c. of the secretion was used, diluted to 80 c.c. during clearing and the volume doubled in the final acetate filtrate. With urine it is 2 since each specimen is diluted but twice. With stool the factor is always 8. In each case, of course, the value is always multiplied according to the amount of the daily specimen.

Separation of Urobilin.

Urine.—To a portion (25 c.c.) of the 24-hour specimen made neutral or barely acid to litmus, about a gram of dry zinc acetate is added and sufficient alcoholic solution of zinc

acetate to make the volume 50 c.c. Shake and filter till clear. A drop or two of tincture of iodine is added to oxidize all the urobilinogen to urobilin and the further treatment is as indicated above.

Stool.—The total stool is agitated with water immediately after collection until a finely divided emulsion is secured.

Dilutions of 250, 500, 750, and 1,000 c.c. are made in accordance with the amount of the specimen and the estimated urobilin content.

Aliquot portions (25 c.c.) are shaken with 75 c.c. of acid alcohol (95 per cent alcohol, 1,600 c.c., conc. HCl 25 c.c. and water to make 2,500 c.c.) in a mechanical shaker for one hour. The specimen is then allowed to stand overnight to complete oxidation.

The next day the mixture is shaken and 25 c.c. portions are taken, about a gram of dry zinc acetate added and alcoholic solution of zinc acetate solution added to make the volume 50 c.c. Shake, filter till clear, and add a drop or two of tincture of iodine. The further treatment is as indicated above.

Bile.—To 20 c.c. add 20 c.c. of 30 per cent ferric chloride solution and 40 c.c. of 20 per cent ammonia water. Filter into a 50 c.c. graduated flask or tube until 25 c.c. are obtained. To this amount of filtrate add concentrated HCl until barely acid to litmus, add about a gram of dry zinc acetate and make up to 50 c.c. with alcoholic solution of zinc acetate. The further treatment is as above.

As the standard fluorescent solution has the same value as 1 mg. of urobilin in 950 c.c., if all dilution values are divided by 950, urobilin content can be expressed in terms of mg. of urobilin.

These methods were devised for experimental work on dogs and their possible clinical application is suggested.

O'Leary, P. A., Goeckerman, W. H., and Parker, S. T.: Treatment of Neurosyphilis by Malaria. Arch. Dermat. and Syph., March, 1926, xiii, No. 3, p. 301.

A preliminary report of a ten months' trial of the method in a series of 35 patients.

Of 24 paretics, 25 per cent had a complete remission two months after the malaria was stopped, 37 per cent were definitely improved, and two died, one as a result of malaria.

There were no serologic changes in the blood or spinal fluid nor any improvement in the objective findings. Optic atrophy, gastric crises, lightning pains, asymptomatic neurosyphilis, and paresis sine paresi were unaffected.

The use of arsenicals, mercury, or tryparsamide immediately after the malaria was attended by relapse from remission.

The relation of the method to public health, the various complications and the methods of forecasting these and so on are discussed.

The authors believe the method merits exhaustive investigation.

Goodpasture, E. W.: A Study of Rabies with Reference to a Neural Transmission of the Virus in Rabbits, and the Structure and Significance of Negri Bodies. Am. Jour. Pathology, November, 1925, i, No. 6, p. 547.

The following technic was used for the demonstration of Negri bodies:

1. Fix fresh tissues in Zenker's fluid for 24 hours. The method is not applicable to tissues fixed in Helly's fluid.

2. Stain sections cut from paraffin 10 minutes in the following solution:

Alcohol 20%	100.0 c.c.
Phenol (pure)	1.0 c.c.
Anilin oil	1.0 c.c.
Basic fuchsin	0.5 gm.

The finely powdered or granular dye dissolves easily and the solution is immediately ready for use. Exposure to light and air causes a precipitation of the dye in about two months so that the solution becomes weaker and must be discarded. It is convenient to

keep the required alcoholic solution of fuchsin, and to add the phenol and anilin oil when it is desired to make up the stain anew. If sections are stained in the solution for an hour or more they gradually become brown and useless. It is safe to stain the sections as long as one-half hour but the best results are obtained within ten minutes.

3. Wash off the excess stain in running water, blot with filter paper and decolorize in 95 per cent alcohol until the sections become pink.

4. Wash off in water and counterstain 15 to 60 seconds with Loeffler's methylene-blue.

5. Wash in water. Dehydrate and decolorize for a few seconds in absolute alcohol (until the excess blue is removed).

6. Clear in xylol; mount in balsam.

By this method Negri bodies are stained a sharp, bright red against a pale blue background.

"InnenKörper" are blue, nucleoli, fibrin, and red blood cells are red and Nissl substance blue.

The smallest forms of Negri bodies are seen.

The investigation demonstrated that:

1. Rabbits can be infected fairly regularly by the injection of street virus into the right masseter muscle.

2. Paralysis first occurs in the fore legs and more often on the inoculated side.

3. It is believed that the virus enters the central nervous system through nerves supplying the inoculated muscle.

4. "Lyssa bodies" seem to be formed directly from a digestive change in the neurofibrillar substances of ganglion cells.

5. Typical Negri bodies seem to be formed within the living cell by a focal degeneration of neurofibrillar material which coheres about one or more of the structures resulting from mitochondrial degeneration.

6. The type of degeneration of mitochondrial substance in axis-cylinders and nerve cells the formation of small bodies staining deeply with Loeffler's methylene-blue after acid fuchsin—is, apparently, specific for rabies.

7. It is believed that neither the virus of rabies nor a constant structural change associated with its pressure has as yet been demonstrated microscopically.

8. The hypothesis is advanced that the degenerative changes observed in axis-cylinders are the result of the passage of the virus along these processes and indicate its manner of extension from one to another.

The material resulting from the degeneration of mitochondria may serve as a favorable medium for the growth and spread of the virus.

The paper is illustrated with five colored plates.

Mill, C. A.: Determination of Small Amounts of Bismuth in the Urine. The Lancet, London, Dec. 19, 1925, ccix, 1281.

The modern therapeutic use of bismuth and its compounds has rendered its detection in the urine of interest and a method has been devised for this purpose by Mill.

1. Destruction of Organic Matter.

Into three separate 100 c.c. silica beakers measure 5, 20, and 50 c.c. of urine and evaporate on an asbestos mat over a Bunsen flame to about 5 c.c.

Remove the beakers and add to each 5 c.c. of nitric acid, (sp. gr. 1.4) replace over the flame and evaporate to dryness, using as low a flame as possible. Continue the heating with a low flame until the organic matter is oxidized but not charred. It is important to stop the ignition when all the nitric acid has been driven off and to cool and moisten again with nitric acid. If necessary the procedure may be repeated until only a white residue remains.

After cooling, add 2 drops of nitric acid and 25 c.c. of distilled water and heat to boiling for one or two minutes and transfer the solution to a Nessler cylinder. Rinse out

the beaker with boiling water, transfer the rinsings to the cylinder and make up to 50 c.c. with boiling water.

The solution should be clear and colorless and filtration will not be necessary if a low temperature has been maintained during the destruction of organic matter.

2. Determination of Bismuth.

To the cooled solution in the Nessler cylinder add 0.3 gm. of urea, dissolve and mix thoroughly, and then add 0.5 gm. of phenazone (antipyrin). Dissolve by shaking and, while shaking, add small crystals (0.05 gm. weight) of potassium iodide one at a time until a red color develops. Then add one crystal in excess.

The color thus produced (due to bismuth iodide) is compared to that of a standard solution prepared as follows:

Dissolve 0.62 gm. of bismuth carbonate (B.P.) in 10 c.c of nitric acid (sp. gr. 1.4) and dilute to 1000 c.c. with distilled water, ten c.c. of this solution is then diluted to 1000 c.c. with distilled water and constitutes the standard.

In the determination, to 10 c.c. of the standard solution (equivalent to 0.05 mg. of bismuth) add 1 drop of nitric acid and dilute to 50 c.c. in a Nessler cylinder. The remaining steps are identical with those of the unknown.

The three unknowns are matched with the standard. The one matching contains in the original amount of urine, 0.05 mg. of bismuth.

McMaster, P. D., and Elman, R.: Studies on Urobilin Physiology and Pathology. II. Urobilin Derivation. Jour. Exper. Med., April, 1925, xli, No. 4, p. 513.

Experiments upon the delivery of bile to the intestine.

Experiments upon dogs led to the conclusion that the existence of urobilin in the stool and bile depends upon the delivery of bile to the intestine.

Urobilin can be obtained from bilirubin by reduction through the action of intestinal bacteria, notably B. putrificus.

The experiments reported indicate that there is a direct absorption of urobilin from the intestine and that it is secreted into the bile through the liver. Urobilin is not directly derived from bleeding into the bowel or tissues.

Mussey, R. D.: Eclampsia, with Unusual Nonprotein Nitrogen in the Blood. Am. Jour. Obst. and Gynec., December, 1925, x, 6.

A case report of a patient, aged twenty-seven, with the clinical symptoms of eclampsia in whom the following blood chemistry was found:

Third day:

Urea	168.0 mg.
Uric acid	18.3 mg.

Fifth day:

Urea	221.0 mg.
Creatinine	14.5 mg.
Uric Acid	14.5 mg.

On the seventeenth day the findings had returned to normal and two months later all tests for renal function were normal.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Surgical Pathology**

IN this volume the authors have attempted to supply a comprehensive survey of surgical pathology in which the embryology, anatomy, pathology, and clinical observations are correlated.

The discussion of each subject follows this plan very successfully and, though brief and to the point, is thorough, so that a large amount of material is included.

The illustrations are taken from specimens in the museum of the Royal College of Surgeons and that of the Charing Cross Hospital Medical School. They are well selected.

The style is clear and readable, and the treatment of the various topics is concise but not ambiguous. The typography and proof reading are excellent.

The volume is primarily a textbook, a dictionary of surgical pathology, and, as such, is a very excellent reference work.

Determinative Bacteriology†

THIS is the second edition of the manual published under the editorial direction of the committee on Determinative Bacteriology of the Society of American Bacteriologists.

This volume needs no introduction to the bacteriologist or clinical pathologist, the first edition having become established as a necessity in any laboratory concerned with bacteriologic examinations.

The revision has been extensive and thorough. The keys have been rearranged and amplified; new species have been added, and many descriptions have been expanded.

Not the least valuable portion of the book is an extensive and excellent cross reference index, including both old and new names, compiled by Robert S. Breed of the New York Agricultural Experiment Station 1.

So numerous and varied have been the changes in bacteriologic nomenclature that this comprehensive index is a valuable and welcome addition to the book. The lack of such an index was felt in the first edition.

*Surgical Pathology. By C. J. Marshall, Lecturer on Surgical Pathology, Charing Cross Hospital Medical School, and A. Piney, Director, Institute of Pathology, Charing Cross Hospital. 173 illustrations. Pp. 478. Price $7.50. D. Appleton and Co., New York.

†Manual of Determinative Bacteriology, a Key for the Identification of Organisms of the Class Schizomycetes. By David H. Bergey, Professor of Bacteriology, University of Pennsylvania. With a Cross Reference Index of Old and New Names by Robert S. Breed. Cloth. 450 pages. Price $5.50. Williams and Wilkins Co., Baltimore.

As it stands, this is a volume which should be purchased for the daily use of laboratories and bacteriologists. There is none which can successfully supplant it in the particular field which it covers.

It is not intended to present the final word in bacteriologic classification, which, to some extent is now, and must remain for a time, in a state of flux, but it serves as an authoritative presentation of the information thus far available and of practical value, and it lends itself to the evolution of a more satisfactory classification in the future.

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*Intravenous Therapy**

AS THE title implies, this book includes a discussion of all therapeutic efforts brought to bear upon the blood stream. It is presented by the author as a manual of intravenous therapy.

The first section of two hundred pages, after an interesting outline of the history of intravenous therapy, is devoted to a detailed discussion of the technic and indications for saline infusion and the transfusion of blood, and this part of the book provides a comprehensive and useful manual of these procedures.

The remainder of the book is concerned entirely with the indications for intravenous medication, diseases being arranged in alphabetical order.

The immediate impressions gained from a perusal of this section of Dr. Dutton's work are: first, that he is an ardent proponent of the intravenous administration of therapeutic agents, almost, it would seem, to the exclusion of other methods; second, that he has assiduously searched the literature for references to substantiate this cause; and third, that, in the presentation of the material thus gathered, there is no sharp distinction between that which *has been* or *might be* administered intravenously and those agents which it is desirable so to administer. As a consequence, there are few diseases known to man which do not appear in the list presented and for which intravenous treatment is not advocated.

One notes the intravenous use of trypsinized extracts of neoplastic tissue in carcinoma; and the statement that by the injection of a hexymethylenamine solution "formaldehyde is at once liberated in the blood, which, as it is a vigorous and powerful antiseptic, destroys the pathogenic germs wherever they may lurk." This is hardly in accord with the usual conception that dissociation of formaldehyde from this drug requires an acid medium. Other similarly somewhat unconsidered statements are to be found here and there under various headings.

The references consulted are, indeed, numerous; too many, however, relate to a series of "one consecutive case," and the author grants, in his preface, that many come from sources of little authority.

It is unfortunate, also, that references to the use of remedies of unknown composition and known only by trade names occur not infrequently.

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*Intravenous Therapy. By W. F. Dutton, Formerly Medical Director, Polyclinic and Medico-Chirurgical Hospitals; Director, Medical Research Laboratories, Amarillo, Texas. Ed. 2. Pp. 594. 64 illustrations. Cloth. Price $6.00. F. A. Davis Co., Philadelphia.

Therapeusis has, at times, been the creature of the winds of chance. It would be regrettable if this book were to serve as the instigation or warrant for the indiscriminate intravenous administration of remedies.

As a source of reference concerning the applications—scientific, empirical, or experimental—of intravenous therapy the book serves a useful purpose, but it must be taken as a record of what has been done or may be done rather than as a definite statement as to what *should* be done in the field of intravenous treatment of disease.

The second portion of the book cannot be accepted as authoritative or as representative of the accepted medical thought of the day and must be read with a full comprehension of the fact that it represents empirical rather than accepted methods based upon sufficient, comprehensive, or scientific investigations.

It is clearly an attempt to make out a case in which the facts are fitted to the theory rather than the reverse.

---

*The Cerebrospinal Fluid in Clinical Diagnosis**

THIS book is an attempt to present in compact form "all that is definitely known about the nature of the spinal fluid and its variations in disease."

After a clearly presented consideration of the anatomy and physiology, followed by a discussion of the nature and composition of the spinal fluid, the authors proceed to a discussion of the various changes which occur in disease. This constitutes Part I of the book.

Part II discusses the spinal fluid findings in meningitis, syphilis, various nervous diseases, and some points of differential diagnosis.

Part III comprises the technic of various methods of examination.

While not exhaustive, the text is well written and informative. The volume serves a useful purpose as a reference work in this field of special pathology, but is neither comprehensive nor cosmopolitan enough in its references to present an all-inclusive survey of the field it covers.

The experience of the authors is amply reflected in the manner of presentation, and the second portion of the book, concerned with the significance and interpretation of spinal fluid findings, should be of great value to the practitioner.

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*The Cerebrospinal Fluid In Clinical Diagnosis. By J. G. Greenfield, Pathologist to The National Hospital for The Paralysed and Epileptic, and E. A. Carmichael, Resident Medical Officer. Pp. 272. 8 illustrations. Cloth. $5.00. Macmillan and Co.

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EDITORIALS

The Respiratory Hormone

STARTING with the work of Haldane and his school widespread interest has in recent years been taken in the nature of the respiratory hormone, that is, in the nature of the chemical changes occurring in the respiratory center which influence the rate and depth of breathing. Since it is known that hyperpnea may be brought about by (*a*) an increase in the CO_2-tension, (*b*) a decrease in the O_2-tension (anoxemia) and (*c*) an increase in P_H of the arterial blood, each of these has in turn been designated as the respiratory hormone, but recent work, especially by Gesell and his collaborators, has shown that no one of them can be the actual respiratory hormone; all three are related to, but none is *the* respiratory hormone. What may this be? Of the three changes the one that is least related to breathing is the P_H of the blood, a fact which has recently been most strikingly demonstrated by Gesell who has devised a method by which it is possible to make continuous observations of the P_H of the arterial blood in a living animal, by

inserting a manganese dioxide *electrode* in a blood vessel. With McGinty he has found that hyperpnea may occur when there is an actual increase in P_H (i.e., decreased acidity) such as following the intravenous injection of solutions of sodium bicarbonate, in anoxemia, or as result of hemorrhage. There is, therefore, no close relationship between hyperpnea and the reaction of the arterial blood. A somewhat closer parallelism exists between hyperpnea and anoxemia, but this does not carry us far in explaining the nature of the hormone, for it is unlikely that a deficiency of O_2 *per se* could act as a stimulus, and it is really only begging the question to state that lack of oxygen increases the sensitivity of the center towards the H-ion; the question remains as to what the actual stimulus may be. Changes in the third factor, the tension of CO_2 of the arterial blood, is perhaps the one which most closely parallels respiratory activity, but even in this case the relationship is not sufficiently pronounced to warrant our considering the arterial CO_2 as the respiratory hormone. For example, although a quantitative increase occurs in breathing when the alveolar CO_2 is raised, as by breathing in CO_2-rich atmospheres, a low arterial CO_2 tension occurs in the hyperpnea due to anoxemia.

Gesell has satisfactorily brought into harmony all these discordant hypotheses by pointing out that *the true respiratory hormone is the H-ion concentration of the respiratory center itself.* This will depend upon several conditions of which the following are the most important: (1) the oxidative processes in the protoplasm of the nerve cells of the center; (2) the ability of the blood circulating through the center to supply it with oxygen and to carry away the products of its metabolism; (3) the exchange of H-ions between the blood and the center; (4) the buffer influence of the protoplasm of cells of the center. With regard to the first of these conditions, it may be supposed that lactic acid will be formed and removed in the nerve cells, just in the same way as in muscle cells. Evidence that this occurs has been obtained by Gesell and McGinty by determinations of the amount of lactic acid which accumulates in brain tissue in the first few minutes following death. The results were similar to those obtained in muscle. It is true that it has been impossible to demonstrate, by comparison of the arterial and venous blood of the brain, that the O_2 consumption is a large one, but, considering the large volume flow of blood as a whole, as well as the fact that only relatively few centers are likely to be active at any one time, the relatively low oxygen consumption is not to be wondered at. The well-known great vulnerability of nerve cells to lack of oxygen on the other hand, is evidence that oxidative processes must be taking place constantly in them.

The second factor comes into play partly through changes in the O_2 tension of arterial blood and partly through changes in the volume flow of blood. When this is sluggish, for example, sufficient O_2 will not be furnished the center, however saturated with this gas the arterial blood may be, nor will lactic acid and CO_2 be removed from it. This is well illustrated in the effect of hemorrhage and subsequent transfusion, breathing being excited

by the former and depressed by the latter. It is significant also that P_H of the arterial blood decreases following hemorrhage and increases following transfusion.

The third factor involves several subsidiary ones of which may be mentioned: (*a*) the P_H of the circulating blood; (*b*) the relative rates of diffusion of ions between the blood and the nerve cells. Of course, the higher the P_H of the blood the higher will tend to be the P_H of the center, other things being equal, but, as we have seen, it is possible for considerable differences in this regard to become established within and without the cell wall. Besides the experiments of Jacobs, Gesell, etc., referred to, Gesell and Hertzman have shown, by their method of continuous measurement of P_H, that when CO_2 was administered the acidity of the arterial blood rose considerably more than that of the venous, indicating that CO_2 must have diffused into the tissue cells. On the other hand when bicarbonate solutions were injected the changes in P_H were practically the same in arterial and venous blood, indicating that the cells had failed to absorb the bicarbonate. These facts indicate that carbonic acid will readily penetrate into the cells of the respiratory center with the result that the P_H of the contents becomes raised and breathing stimulated, whereas bicarbonate will fail to pass into them, so that breathing will be relatively unaffected.

The fourth factor is difficult to appraise. It is probable that the buffer influence of the cells is much lower than that of the blood so that changes in P_H will readily occur and it is also to be remembered that when lactic acid is formed it will affect the P_H of the center relatively more than an equivalent quantity of CO_2, because of its lower diffusibility through the cell wall. —*J. J. R. M.*